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2. RDDR MODELING

The Regional Deposited Dose Ratio (RDDR) is the ratio of the deposited dose in a respiratory tract region of interest for the laboratory animal species (RDD_A) relative to the deposited dose for humans (RDD_H). This ratio is used to adjust the measured or nominal particulate exposure level for inter-species dosimetric differences in the various regions of the respiratory tract (*i.e.*, pulmonary [PU], extra-thoracic [ET], tracheobronchial [TB], thoracic [PU + TB], total respiratory tract [RT], and extra-respiratory [ER] regions). For each of the surfactants with available animal toxicity studies, RDDRs were calculated according to the procedures in EPA's "*Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*" (EPA, 1994). The RDDRs were used as Dosimetric Adjustment Factors (DAFs) to adjust the animal exposure concentrations to human equivalent concentrations (HEC).

For surfactants, it is expected that the deposited dose in the various regions of the respiratory tract correlate with adverse outcomes, thus the RDDR value is appropriate for surfactant inhalation assessments. The input parameters for the RDDR calculations are based on the characteristics of the aerosol tested in the inhalation study (Median Mass Aerodynamic Diameter or MMAD, Geometric Standard Deviation or GSD), human body mass, animal species, animal mass (which varies by gender), *etc*. The input parameters and resulting RDDRs calculated for each respiratory tract region are summarized in Supplemental [REF _Ref47673451 \h]. The lowest RDDR value was selected as the DAF as per EPA Guidance and used to derive HECs as shown in Table 3 of the manuscript.

The RDDR values and software outputs for the surfactants octylphenoxypolyethoxyethanol, oleoyl sarcosine, didecyl dimethylammonium chloride (DDAC), and benzalkonium chloride (BAC) are provided in **Figures 3-10**. The RDDR outputs were calculated separately for male and female rats to account for differences in body weights as per RDDR program guidance. Rat body weights were derived from the individual studies and adult human default body weight used was 80 kilograms. Other inputs to the RDDR program are default values and are listed in each figure.

Table [SEQ Table * ARABIC]. RDDR input parameters and calculated values.

Surfactant Type	Chemical Substance	Inhalation Exposure Duration/ Type 14-day, 6 hr/d, 5 d/wk; whole body	Density (g/cm^3) at 20°C **	RDDR Model Input Parameters		RDDR									
						Extra-Thoracic (ET)		Tracheo-bronchial (TB)		Pulmonary (PU)		Thoracic (PU+TB)		Total (RT)	
				MMAD (μm)	GSD	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Nonionic	octylphenoxy polyethoxyethanol (CASRN 9002-93-1)	28-day, 6 hr/d, 5 d/wk; nose-only (OECD TG 412)	0.998 water vehicle	1.80	1.80	0.351	0.196	1.459	1.367	0.564	0.610	0.812	0.823	2.432	1.547
Anionic	oleoyl sarcosine (CASRN 110-25-8)	4-week, 6 hr/d, 5 d/wk; nose-only	0.7893 ethanol vehicle	1.16	2.12	0.210	0.111	2.457	2.008	0.470	0.447	0.844	0.742	1.504	0.970
Cationic	DDAC 28-day Study	14-day, 6 hr/d, 7 d/wk; whole body	NR	1.60	1.85	0.410	0.211	1.855	1.674	0.539	0.583	0.861	0.854	2.692	1.607
	BAC	Inhalation Exposure Duration/ Type	0.998 water vehicle, 1-2% dose solution	1.31	1.79	0.184	0.106	2.307	1.998	0.557	0.528	0.899	0.815	1.414	0.991

i. Octylphenoxypolyethoxyethanol RDDR Results

Regional deposited dose ratios

MMAD = 1.80
Sigma g = 1.80

SPECIES	Body weight(g)	VE(ml)	Extrathoracic SA(cm^2)	dep	Tracheobronchial SA(cm^2)	dep	Pulmonary SA(m^2)	dep
rat	332	226.9	15.000	0.507	22.500	0.049	0.340	0.056
human	80000	13800.0	200.000	0.317	3200.000	0.079	54.000	0.258
RATIO	0.004	0.016	0.075	1.599	0.007	0.624	0.006	0.216
RDDR			0.351		1.459		0.564	

	Thoracic SA(m^2)	dep	Total RT SA(m^2)	dep	Extrarespiratory BW(g)	dep
rat	0.342	0.105	0.344	0.611	332	0.611
human	54.320	0.125	54.340	0.654	80000	0.654
RATIO	0.006	0.837	0.006	0.936	0.004	0.936
RDDR	0.812		2.432		3.707	

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Figure 3. Octylphenoxypolyethoxyethanol RDDR Results for Male Rats.

Regional deposited dose ratios

MMAD = 1.80
Sigma g = 1.80

SPECIES	Body weight(g)	VE(ml)	Extrathoracic SA(cm^2)	dep	Tracheobronchial SA(cm^2)	dep	Pulmonary SA(m^2)	dep
rat	209	155.2	15.000	0.413	22.500	0.067	0.340	0.088
human	80000	13800.0	200.000	0.317	3200.000	0.079	54.000	0.258
RATIO	0.003	0.011	0.075	1.304	0.007	0.855	0.006	0.341
RDDR			0.196		1.367		0.610	

	Thoracic SA(m^2)	dep	Total RT SA(m^2)	dep	Extrarespiratory BW(g)	dep
rat	0.342	0.155	0.344	0.569	209	0.569
human	54.320	0.125	54.340	0.654	80000	0.654
RATIO	0.006	1.240	0.006	0.870	0.003	0.870
RDDR	0.823		1.547		3.745	

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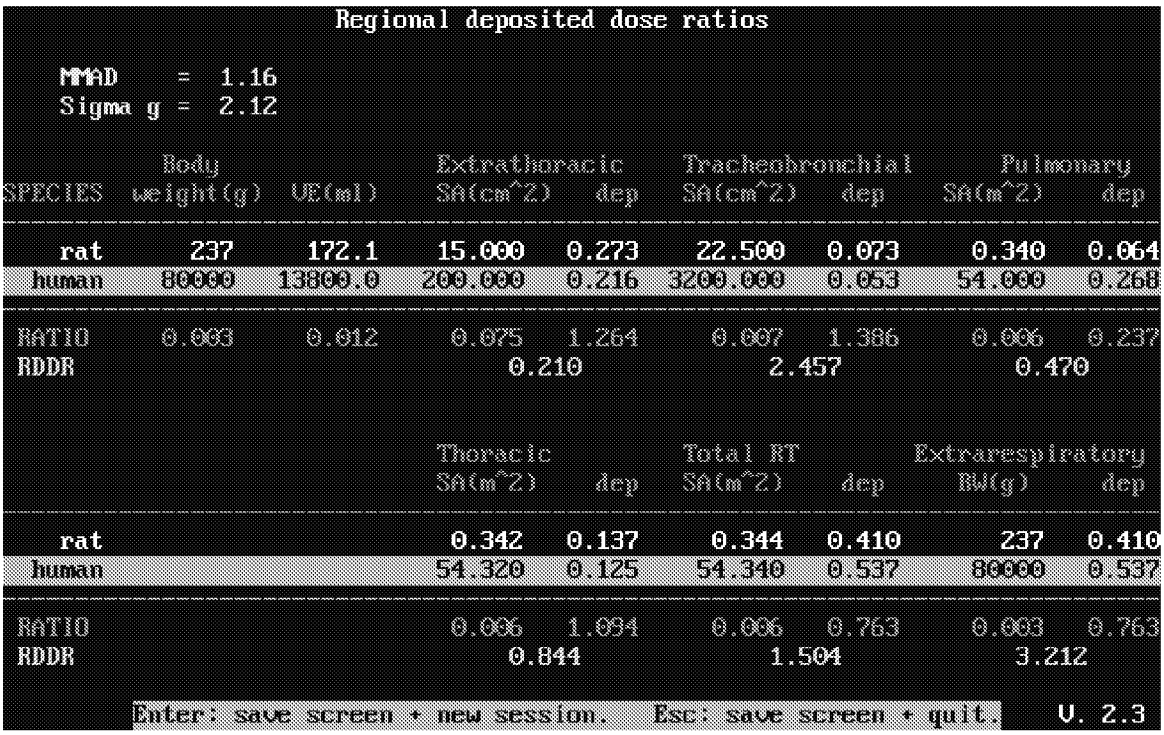
Figure 4. Octylphenoxypolyethoxyethanol RDDR Results for Female Rats.

ii. Oleoyl sarcosine RDDR Results



Regional deposited dose ratios

MMAD = 1.16
Sigma g = 2.12

SPECIES	Body weight(g)	VE(ml)	Extrathoracic SA(cm^2)	dep	Tracheobronchial SA(cm^2)	dep	Pulmonary SA(m^2)	dep
rat	237	172.1	15.000	0.273	22.500	0.073	0.340	0.064
human	80000	13800.0	200.000	0.216	3200.000	0.053	54.000	0.268
RATIO	0.003	0.012	0.075	1.264	0.007	1.386	0.006	0.237
RDDR			0.210		2.457		0.470	

	Thoracic SA(m^2)	dep	Total RT SA(m^2)	dep	Extrarespiratory BW(g)	dep
rat	0.342	0.137	0.344	0.410	237	0.410
human	54.320	0.125	54.340	0.537	80000	0.537
RATIO	0.006	1.094	0.006	0.763	0.003	0.763
RDDR	0.844		1.504		3.212	

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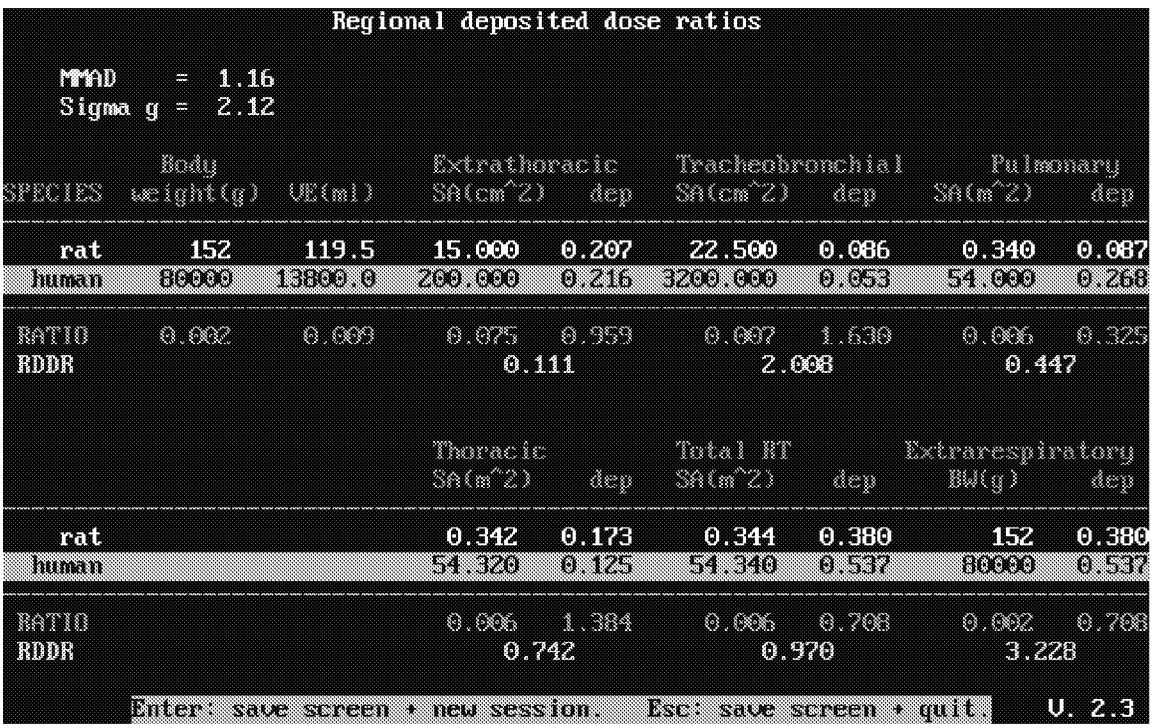
Figure 5. Oleoyl sarcosine RDDR Results for Male Rats.



Regional deposited dose ratios

MMAD = 1.16
Sigma g = 2.12

SPECIES	Body weight(g)	VE(ml)	Extrathoracic SA(cm^2)	dep	Tracheobronchial SA(cm^2)	dep	Pulmonary SA(m^2)	dep
rat	152	119.5	15.000	0.207	22.500	0.086	0.340	0.087
human	80000	13800.0	200.000	0.216	3200.000	0.053	54.000	0.268
RATIO	0.002	0.009	0.075	0.959	0.007	1.630	0.006	0.325
RDDR			0.111		2.008		0.447	

	Thoracic SA(m^2)	dep	Total RT SA(m^2)	dep	Extrarespiratory BW(g)	dep
rat	0.342	0.173	0.344	0.380	152	0.380
human	54.320	0.125	54.340	0.537	80000	0.537
RATIO	0.006	1.384	0.006	0.708	0.002	0.708
RDDR	0.742		0.970		3.228	

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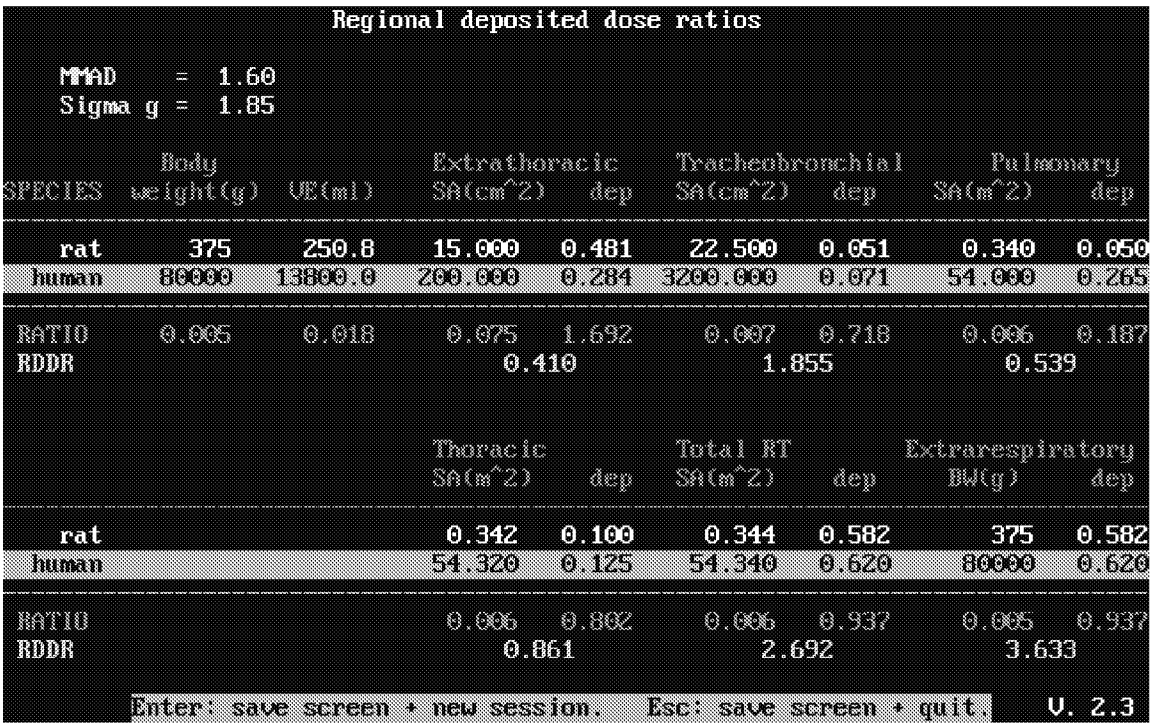
Figure 6. Oleoyl sarcosine RDDR Results for Female Rats.

iii. DDAC RDDR Results



Regional deposited dose ratios

MMAD = 1.60
Sigma g = 1.85

SPECIES	Body weight(g)	VE(ml)	Extrathoracic SA(cm^2)	dep	Tracheobronchial SA(cm^2)	dep	Pulmonary SA(m^2)	dep
rat	375	250.8	15.000	0.481	22.500	0.051	0.340	0.050
human	80000	13800.0	200.000	0.284	3200.000	0.071	54.000	0.265
RATIO	0.005	0.018	0.075	1.692	0.007	0.718	0.006	0.187
RDDR			0.410		1.855		0.539	

	Thoracic SA(m^2)	dep	Total RT SA(m^2)	dep	Extrarespiratory BW(g)	dep
rat	0.342	0.100	0.344	0.582	375	0.582
human	54.320	0.125	54.340	0.620	80000	0.620
RATIO	0.006	0.802	0.006	0.937	0.005	0.937
RDDR	0.861		2.692		3.633	

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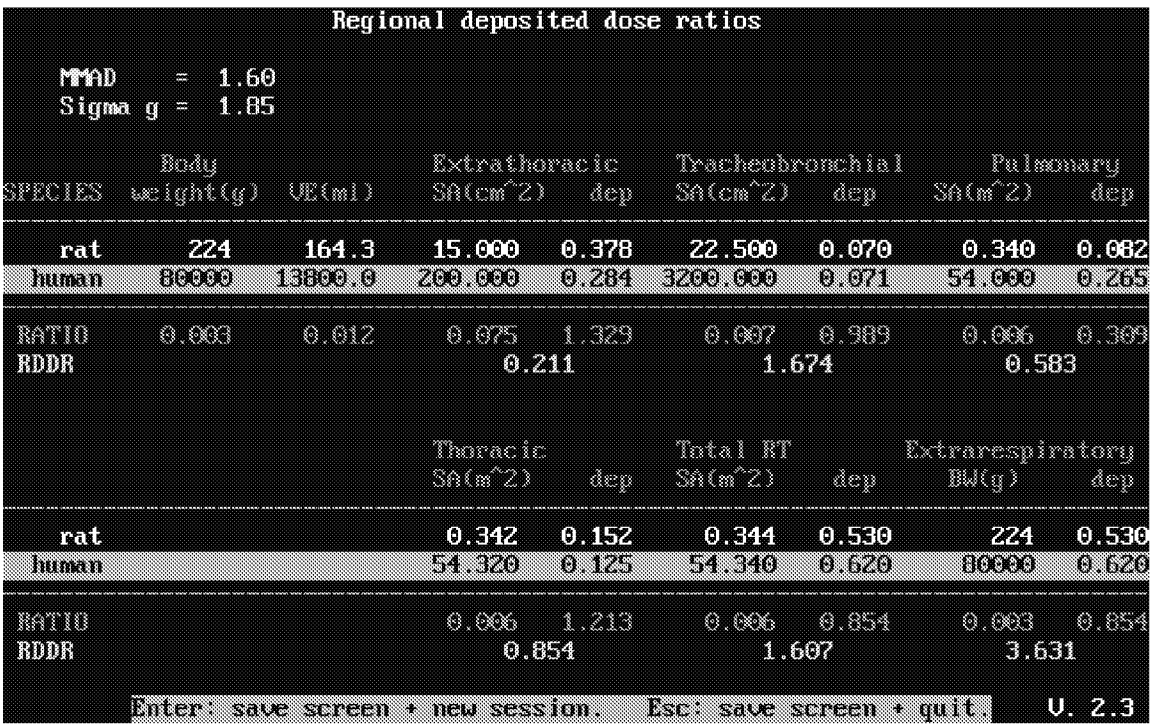
Figure 7. DDAC RDDR Results for Male Rats in the 28-Day Inhalation Study.



Regional deposited dose ratios

MMAD = 1.60
Sigma g = 1.85

SPECIES	Body weight(g)	VE(ml)	Extrathoracic SA(cm^2)	dep	Tracheobronchial SA(cm^2)	dep	Pulmonary SA(m^2)	dep
rat	224	164.3	15.000	0.378	22.500	0.070	0.340	0.082
human	80000	13800.0	200.000	0.284	3200.000	0.071	54.000	0.265
RATIO	0.003	0.012	0.075	1.329	0.007	0.989	0.006	0.309
RDDR			0.211		1.674		0.583	

	Thoracic SA(m^2)	dep	Total RT SA(m^2)	dep	Extrarespiratory BW(g)	dep
rat	0.342	0.152	0.344	0.530	224	0.530
human	54.320	0.125	54.340	0.620	80000	0.620
RATIO	0.006	1.213	0.006	0.854	0.003	0.854
RDDR	0.854		1.607		3.631	

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Figure 8. DDAC RDDR Results for Female Rats in the 28-Day Inhalation Study.

iv. BAC RDDR Results

Regional deposited dose ratios

MMAD = 1.31
Sigma g = 1.79

SPECIES	Body weight(g)	VE(ml)	Extrathoracic SA(cm^2)	dep	Tracheobronchial SA(cm^2)	dep	Pulmonary SA(m^2)	dep
rat	207	154.0	15.000	0.281	22.500	0.083	0.340	0.088
human	80000	13800.0	200.000	0.227	3200.000	0.057	54.000	0.280
RATIO	0.003	0.011	0.075	1.239	0.007	1.454	0.006	0.314
RDDR			0.184		2.307		0.557	

	Thoracic SA(m^2)	dep	Total RT SA(m^2)	dep	Extrarespiratory BW(g)	dep
rat	0.342	0.171	0.344	0.452	207	0.452
human	54.320	0.125	54.340	0.564	80000	0.564
RATIO	0.006	1.369	0.006	0.801	0.003	0.801
RDDR	0.899		1.414		3.456	

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Figure 9. BAC RDDR Results in Male Rats.

Regional deposited dose ratios

MMAD = 1.31
Sigma g = 1.79

SPECIES	Body weight(g)	VE(ml)	Extrathoracic SA(cm^2)	dep	Tracheobronchial SA(cm^2)	dep	Pulmonary SA(m^2)	dep
rat	145	115.0	15.000	0.216	22.500	0.096	0.340	0.112
human	80000	13800.0	200.000	0.227	3200.000	0.057	54.000	0.280
RATIO	0.002	0.008	0.075	0.955	0.007	1.678	0.006	0.399
RDDR			0.106		1.988		0.528	

	Thoracic SA(m^2)	dep	Total RT SA(m^2)	dep	Extrarespiratory BW(g)	dep
rat	0.342	0.208	0.344	0.425	145	0.425
human	54.320	0.125	54.340	0.564	80000	0.564
RATIO	0.006	1.662	0.006	0.752	0.002	0.752
RDDR	0.815		0.991		3.459	

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Figure 10. BAC RDDR Results in Female Rats.

Message

From: Stedeford, Todd [Stedeford.Todd@epa.gov]
Sent: 8/18/2020 1:20:27 PM
To: Sahar_Osman-Sypher@americanchemistry.com; Henry, Tala [Henry.Tala@epa.gov]; Salazar, Keith [Salazar.Keith@epa.gov]; Irwin, William [Irwin.William@epa.gov]
Subject: Surfactants --> 18 August 2020.ver.1 (latest draft)
Attachments: draft manscript general surfactants - 18 August 2020.ver.1.docx

Importance: High

All, here is the latest draft. I incorporated the text edits from Raphael and updated the DDAC language (minor edits), all of which are in track changes.

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Surfactants Category: The Application of a New Approach Methodology (NAM) for Assessing Inhalation Risks under the Amended Toxic Substances Control Act

Commented [HT1]: NOTE: EPA's Strategy AND the Tiered Testing Strat in the paper uses multiple assays, so this should be plural

BUT this is now different from the LO paper, so keep or change?

Commented [ST2R1]: The idea is that the tiered-testing strategy is the NAM, not the individual assays

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KEYWORDS: Inhalation, Surfactant, New Approach Methodologies, Lung Toxicity, Risk Assessment

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ABSTRACT

The Toxic Substances Control Act (TSCA) requires anyone who plans to manufacture (including import) a new chemical substance for a non-exempt commercial purpose to provide the U.S. Environmental Protection Agency (EPA) with a premanufacture notice (PMN) prior to commercialization. Surfactants are a class of chemical substances used in a variety of industrial operations, occupational settings, and in consumer products. Their uses in such applications provide pathways of exposure by which potential toxicity of these compounds may occur to humans. While TSCA requires submission of any existing toxicity data, it does not require

generation of toxicity data for the purpose of, or prior to, submitting a PMN. TSCA requires EPA to review the PMN to determine whether the new chemical substance presents an unreasonable risk of injury to human health or the environment and mandates that EPA reduce or replace vertebrate animals in testing, to the extent practicable and scientifically justified. EPA therefore relies on several approaches that do not rely on *de novo* toxicity testing. Analogue read-across, in which toxicity data for a chemical of similar structure and activity are used to assess the new chemical, and chemical categories (a group of chemicals whose properties are likely to be similar or follow a regular pattern as a result of mechanism, mode of toxic action or structural similarity) have been used by EPA for decades to assess new chemical substances. This investigation was conducted to identify surfactant chemicals with toxicity data relevant for use in conducting a quantitative human health risk assessment for new surfactant substances and to define a TSCA New Chemical Category for surfactants. Category boundaries, which are defined, toxicological analogues suitable for conducting 'read-across' hazard assessment (*i.e.*, hazard identification and dose-response analysis) are identified and a tiered-testing strategy aimed at using new approach methodologies (NAMs) to reduce or replace animal testing is outlined. This tiered strategy to defining and evaluating the Surfactant Category provides a pragmatic and scientifically defensible approach to facilitate EPA's review of PMNs for new surfactants and a strategic testing approach that provides the data needed to conduct or refine surfactant risk assessments while also meeting the requirements of TSCA to reduce vertebrate testing.

Commented [SK4]: Capitalization was inconsistent throughout. I capitalized everywhere.

INTRODUCTION

The Toxic Substances Control Act (TSCA) was amended in 2016 by the Frank R. Lautenberg Chemical Safety for the 21st Century Act (Pub. L. 114-182). The amended TSCA included

substantial changes to EPA's authorities and responsibilities, including requirements on EPA to make a determination regarding sufficiency of information, environmental releases and human exposure, and unreasonable risks. The amended TSCA also included provisions mandating EPA to "reduce and replace, to the extent practicable, [and] scientifically justified" the use of vertebrate animals in the testing of chemicals substances. Specifically, TSCA section 4(h) charges EPA with encouraging and facilitating –

> (1) the use of scientifically valid test methods and strategies that reduce or replace the use of vertebrate animals while providing information of equivalent or better scientific quality and relevance that will support regulatory decisions under TSCA;
> (2) the grouping of 2 or more chemical substances into scientifically appropriate categories in cases in which testing of a chemical substance would provide scientifically valid and useful information on other chemical substances in the category; and
> (3) the formation of industry consortia to jointly conduct testing to avoid unnecessary duplication of tests, provided that such consortia make all information from such testing available to the Administrator.

The present investigation advances each of these TSCA mandates for chemical substances characterized as surfactants.

A surfactant is a substance that reduces the surface tension of a liquid in which it is dissolved. They are surface-active, amphiphilic compounds that self-assemble to form micelles or aggregates above a critical concentration, referred to as the critical micelle concentration (CMC).

These substances are commonly used in industrial processes, occupational settings, and in consumer products (*e.g.*, household cleaning products, personal care products, *etc.*) as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. The widespread use of surfactants provides opportunities for releases and exposure to human or environmental receptors. The inherent properties of surfactants may induce toxicity if exposures can interfere with biological surfactants or tissues. Certain surfactants are commonly used in a laboratory setting to disrupt cell membranes and denature proteins, which demonstrates the inherent hazards of surfactants. For example, sodium dodecyl sulfate (SDS), a strong anionic surfactant, is used at concentrations up to 10% to disrupt cell membranes and to denature proteins, whereas octylphenoxypolyethoxyethanol , a mild nonionic surfactant, at concentrations up to 1% disrupt cell membranes, while preserving proteins for isolation [ADDIN EN.CITE <EndNote><Cite><Author>Burden</Author><Year>2012</Year><RecNum>14727</RecNum><DisplayText>[1]</DisplayText><record><rec-number>14727</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596017177">14727</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Burden, D.W.</author></authors></contributors><titles><title>Guide to the Disruption of Biological Samples - 2012, Version 1.1.</title><secondary-title>Random Primers</secondary-title></titles><periodical><full-title>Random Primers</full-title></periodical><pages>1-25</pages><number>12</number><dates><year>2012</year></dates><urls></urls></record></Cite></EndNote>].

Hazard concerns for surfactants historically focused on their observed environmental effects and potential toxicity to aquatic organisms based on “down the drain” releases and/or presence in effluent from wastewater treatment facilities [ADDIN EN.CITE ADDIN EN.CITE.DATA].The EPA has established chemical categories for nonionic, anionic, and cationic (quaternary ammonium) surfactants based on environmental toxicity concerns [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2010</Year><RecNum>14729</RecNum><DisplayText>[3]</DisplayText><record><rec-number>14729</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596017536">14729</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>TSCA New Chemicals Program (NCP) Chemical Categories</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>157, https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf</pages><dates><year>2010</year></dates><urls></urls></record></Cite></EndNote>]. Surfactants may pose a potential hazard to humans, depending on their use and route of exposure, because they can disrupt the normal architecture of the lipid bilayer and reduce the surface tension, thereby solubilizing cell membranes. Mucous membranes are particularly sensitive to the surface-active effects of surfactants, which have been shown to cause irritancy and injury to the eye, based on their ability to “readily penetrate the sandwiched aqueous and lipid barriers of the cornea” [ADDIN

Commented [HT5]: There is a 2010 Category for all 3 Anionic on pg 34, cationic on pg 51 and nonionic, nonionic on pg 94

The categories were established long before 2010; just updated in 2010.

EN.CITE

<EndNote><Cite><Author>Fox</Author><Year>2008</Year><RecNum>14730</RecNum><DisplayText>[4]</DisplayText><record><rec-number>14730</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596017801">14730</key></foreign-keys><ref-type name="Book Section">5</ref-type><contributors><authors><author>Fox, D.A.</author><author>Boyes, W.K.</author></authors><secondary-authors><author>Klaassen, C.D.</author></secondary-authors></contributors><titles><title>Toxic Responses of the Ocular and Visual System</title><secondary-title>Casarett & Doull's Toxicology - The Basic Science of Poisons, Seventh Edition</secondary-title></titles><pages>665-697</pages><section>17</section><dates><year>2008</year></dates><pub-location>New York</pub-location><publisher>McGraw-Hill, Medical Publishing Division</publisher><urls></urls></record></Cite></EndNote>].

Depending on the conditions of use, the potential for inhalation exposures to workers and/or consumers warrant consideration in quantitative risk assessments. Surfactants may cause adverse effects on mucous membranes, including the respiratory tract, and interfere with the natural pulmonary surfactants and result in reduction in the oxygen content of arterial blood due to impaired gas exchange in the lung, increases in pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, grossly visible pulmonary edema, and atelectasis [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The chemical category boundary for surfactants that may have the potential to present an inhalation hazard has not been previously defined. The toxicity of surfactants by inhalation exposure can vary over several orders of magnitude, based

on their chemical properties. For example, octylphenoxypolyethoxyethanol, (CASRN 9002-93-1) a nonionic surfactant, had a lowest-observed-adverse-effect concentration [LOAEC] of 5.3 mg/m^3) in a 14-day study [ADDIN EN.CITE ADDIN EN.CITE.DATA], while didecyldimethyl ammonium chloride (DDAC; CASRN 7173-51-5), a cationic surfactant and biocide, had a LOAEC of 0.08 mg/m^3 in a 4-week study [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2016</Year><RecNum>14732</RecNum><DisplayText>[10]</DisplayText><record><rec-number>14732</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596018482">14732</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Subchronic Inhalation Toxicity Study of DDAC - Revised</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>25</pages><volume>HQ-OPP-2006-0338-0045</volume><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>] .

The objectives of the present investigation were to: (1) perform a systematic review of the literature with the aim of defining the chemical space for surfactants; (2) identify inhalation toxicity studies on surfactants that may be used to inform inhalation risk assessments; (3) describe scientifically sound new approach methodologies (NAMs) to reduce or replace animal

testing; and (4) establish a tiered-testing strategy that uses NAMs to evaluate new chemistries in the Surfactant Category.

MATERIALS AND METHODS

Systematic Literature Review

Two literature searches were performed, an initial search from 1950 through November 2016 and a supplemental search up to April 2018. The details of these searches, including the search strategies, search terms, search results and Population, Exposure, Comparison, and Outcome (PECO) criteria used for reviewing the relevance of the identified studies to this evaluation are provided in the Supporting Information file at "Section 1 Systematic Literature Review". These searchers were conducted with the primary objective of identifying studies that evaluated the toxicity of surfactants in the respiratory tract of humans or laboratory animals, and at the cellular level in *in vitro* and *ex vivo* studies. In addition, these searches were used to identify potential NAMs that could inform a tiered-testing strategy for general surfactants that reduces or replaces the use of vertebrate animals in regulatory testing.

Risk Assessment Approaches under TSCA

Risk Assessment Paradigm

The methods for assessing risks of new chemical substances under TSCA have been developed using science based approaches, scientific peer review, and refinement of the approaches. EPA conducts risk assessments following the four-step process articulated by the U.S. National Research Council (NRC) in 1983 [11] and reaffirmed several times since its initial release [12, 13]. This process includes hazard identification, dose-response analysis, exposure assessment,

and risk characterization. Hazard assessment (also called effects assessment in some EPA guidance documents) identifies the adverse health or environmental effects, or hazards, that can be caused by exposure to a chemical substance. The dose-response analysis assesses the relationship between the exposure or dose of a chemical and the occurrence of health or environmental effects. The exposure assessment characterizes human or environmental exposures, including the magnitude, frequency, and duration, to the extent necessary and practicable within the context of the assessment. Finally, the risk characterization integrates the hazard, dose-response, and exposure components to describe the nature, and when possible, the magnitude of risks to human health and the environment.

The approaches employed for these risk assessment components, including the level of detail and complexity of quantitative aspects, may vary across different risk assessments and typically align with specific legislative and regulatory frameworks. For example, legislative and regulatory frameworks for hazard evaluation of pesticide active ingredients, anti-microbial substances, inerts, *etc*. are described in regulations for pesticides, which include multiple and specific requirements for toxicity data. Under TSCA and its implementing regulations [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2020</Year><RecNum>14738</RecNum><DisplayText>[11]</DisplayText><record><rec-number>14738</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019129">14738</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>40 CFR Part 720 - Premanufacture

Notification</title><secondary-title>Code of Federal Regulations</secondary-title></titles><periodical><full-title>Code of Federal Regulations</full-title></periodical><pages>https://www.law.cornell.edu/cfr/text/40/part-720</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], companies are required to submit a PMN along with available data on: chemical identity, production volume, byproducts, use, environmental release, disposal practices, and human exposure. These submissions are required to include all existing health and environmental data in the possession or control of the submitter, parent company, or affiliates, and a description of any existing data known to or reasonably ascertainable by the submitter. However, TSCA has never included requirements for toxicity testing or generation of hazard data for new chemical substances.

Hazard Assessment

Given the lack of toxicity testing requirements under TSCA, EPA only occasionally receives hazard data for new chemical substances. An analysis of toxicity data submitted to EPA from 2004 through 2012 for new chemical substances found that only about 15% of the PMN submissions included health hazard data; the majority of that information was for acute toxicity (*e.g.*, oral and/or dermal) and irritation (*e.g.*, eye and/or skin) in animals. TSCA provides EPA with the authority to require generation and submission of additional data when the information included with the PMN— coupled with that available to EPA risk assessors from prediction modeling, read-across, internal archives, *etc.* —is insufficient to permit a reasoned evaluation of the health and environmental effects of a new chemical substance. However, prior to making a request for testing using vertebrate animals, EPA must take into consideration reasonably

available existing information, including toxicity information; computational toxicology and bioinformatics; and high-throughput screening methods and the prediction models of those methods (TSCA Section 4(h)(A)(i)-(iii)).

Given the historical lack of hazard data, EPA has, for decades, employed a number of approaches that do not rely on *de novo* toxicity testing. These approaches include computational toxicology (*e.g.*, predictive models and expert systems), analogue[1] read-across wherein available toxicity data for a chemical of similar structure and activity are used to assess the new chemical substance lacking data, and chemical categories (a group of chemicals whose properties are likely to be similar or follow a regular pattern as a result of mechanism, mode of toxic action or structural similarity) [ADDIN EN.CITE <EndNote><Cite><Author>van Leeuwen</Author><Year>2009</Year><RecNum>14739</RecNum><DisplayText>[12]</DisplayText><record><rec-number>14739</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019290">14739</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>van Leeuwen, K.</author><author>Schultz, T. W.</author><author>Henry, T.</author><author>Diderich, B.</author><author>Veith, G. D.</author></authors></contributors><auth-address>TNO Quality of Life, Utrechtseweg 48, The Netherlands.</auth-address><titles><title>Using chemical categories to fill data gaps in hazard assessment</title><secondary-title>SAR QSAR Environ Res</secondary-title><alt-

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[1] In the context of this article, an analogue is a chemical substance identified based on its physicochemical and toxicological properties, as one that has undergone evaluation, as stated above, and determined to be an acceptable toxicological analogue for read across to the new chemical substance. An analogue may be directly used in read-across for informing a quantitative risk assessment on a new chemical substance.

title>SAR and QSAR in environmental research</alt-title></titles><periodical><full-title>SAR QSAR Environ Res</full-title><abbr-1>SAR and QSAR in environmental research</abbr-1></periodical><alt-periodical><full-title>SAR QSAR Environ Res</full-title><abbr-1>SAR and QSAR in environmental research</abbr-1></alt-periodical><pages>207-20</pages><volume>20</volume><number>3-4</number><edition>2009/06/23</edition><keywords><keyword>Hazardous Substances/pharmacology/*toxicity</keyword><keyword>*Quantitative Structure-Activity Relationship</keyword><keyword>Safety Management/*methods</keyword></keywords><dates><year>2009</year></dates><isbn>1026-776x</isbn><accession-num>19544189</accession-num><urls></urls><electronic-resource-num>10.1080/10629360902949179</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]. The integration of these methods with NAMs to advance testing strategies has been recognized by EPA [ADDIN EN.CITE ADDIN EN.CITE.DATA] and is consistent with the vision articulated in the 2007 report by the NRC in "Toxicity Testing in the 21st Century: A Vision and Strategy" [ADDIN EN.CITE <EndNote><Cite><Author>NRC</Author><Year>2007</Year><RecNum>14741</RecNum><DisplayText>[14]</DisplayText><record><rec-number>14741</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019531">14741</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>NRC</author></authors></contributors><titles><title>T

oxicity Testing in the 21st Century: A Vision and a Strategy, Washington, D.C. The National Academies Press</title></titles><pages>216, DOI: https://doi.org/10.17226/11970</pages><volume>ISBNs: Ebook: 978-0-309-13412-5; Paperback: 978-0-309-15173-3</volume><dates><year>2007</year></dates><urls></urls></record></Cite></EndNote>]. EPA defines NAMs "as a broadly descriptive reference to any technology, methodology, approach, or combination thereof that can be used to provide information on chemical hazard and risk assessment that avoids the use of intact animals" [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2018</Year><RecNum>14844</RecNum><DisplayText>[15]</DisplayText><record><rec-number>14844</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1597332016">14844</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Strategic Plan to Promote the Development and Implementation of Alternative Test Methods within the TSCA Program</title><secondary-title>Office of Chemical Safety and Pollution Prevention & Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention & Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>39, https://www.epa.gov/sites/production/files/2018-06/documents/epa_alt_strat_plan_6-20-18_clean_final.pdf</pages><volume>EPA-740-R1-8004</volume><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>]

[PAGE]

Dose-Response Analysis

In the absence of test data on new chemical substances, EPA relies on read-across methods using an analogue or a category of analogues to identify hazards and conduct dose-response analysis to identify a point of departure (POD), *i.e.*, a dose or concentration that marks the beginning of a low-dose extrapolation) in the absence of test data on the new chemical substance. EPA's "TSCA New Chemicals Program (NCP) Chemical Categories" [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2010</Year><RecNum>14729</RecNum><DisplayText>[3]</DisplayText><record><rec-number>14729</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596017536">14729</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>TSCA New Chemicals Program (NCP) Chemical Categories</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>157, https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf</pages><dates><year>2010</year></dates><urls></urls></record></Cite></EndNote>] for anionic, nonionic, and cationic surfactants were developed and defined only on environmental toxicity considerations. Toxicity data for analogues are used to identify a POD, such as a no observed adverse effect (concentration) level (NOAE(C)L) or lowest observed adverse effect (concentration) level

Commented [ST6]: Seems out of order given first sentence, i.e., an analogue and then category

(LOAE(C)L, for assessing risks of the new chemical substance. This POD can also be the lower bound on dose (or concentration) for an estimated incidence or a change in response level calculated by a dose-response model such as those available in EPA's benchmark dose software (BMDS), *e.g.*, the BMCL for an observed incidence or change in level of response [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2012</Year><RecNum>14744</RecNum><DisplayText>[16]</DisplayText><record><rec-number>14744</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019975">14744</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Benchmark Dose Technical Guidance</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>99, https://www.epa.gov/sites/production/files/2015-01/documents/benchmark_dose_guidance.pdf</pages><volume>EPA/100/R-12/001</volume><dates><year>2012</year></dates><urls></urls></record></Cite></EndNote>].

EPA has also developed guidance to improve the science underlying the animal-to-human uncertainty factor and provides generalized procedures for deriving dosimetric adjustment factors (DAFs) [ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>2002</Year><RecNum>14743</RecNum><DisplayText>[17, 18]</DisplayText><record><rec-number>14743</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019884">14743</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>A Review of the Reference Dose and Reference Concentration Processes</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>192, https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf</pages><volume>EPA/630/P-02/002F</volume><dates><year>2002</year></dates><urls></urls></record></Cite><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389,

https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. Application of DAFs to the animal airborne exposure values yields estimates of the concentration that would result in the same concentration to humans, that is, the human equivalent concentration (HEC). Application of a DAF in the calculation of an HEC is considered to address the toxicokinetic aspects of the animal-to-human uncertainty factor (UF) (*i.e.*, to estimate from animal exposure information the human exposure scenario that would result in the same dose to a given target tissue) (EPA, 2002). This operational derivation involves the use of species-specific physiologic and anatomic factors relevant to the form of pollutant (*e.g.*, particle, reactive gas, or volatile organic compound) and categorized with regard to elicitation of response. These factors are all employed in determining the appropriate DAF. For HECs, DAFs are applied to the "duration-adjusted" concentration to which the animals were exposed (*e.g.*, to a weekly average).

For interspecies extrapolation of particle exposures, the Regional Deposited Dose Ratio (RDDR) model developed by EPA can be used to derive a DAF. The RDDR is the ratio of the deposited dose in a respiratory tract region (r) for the laboratory animal species of interest (RDD_A) to that of humans (RDD_H) [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><DisplayText>[18]</DisplayText><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal

Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. EPA's RDDR model allows calculation of RDDR estimates in various regions of the respiratory tract for animals versus humans (*i.e.*, extra-thoracic [ET], tracheobronchial [TB], pulmonary [PU], thoracic [TH], total respiratory tract [RT] and extra-respiratory [ER] regions). The RDDR calculation is based on the characteristics of the aerosol tested in the inhalation study (Median Mass Aerodynamic Diameter or MMAD, Geometric Standard Deviation or GSD, and density), and species-specific parameters for both animals and humans including ventilation rates and regional surface areas of the respiratory tract. The RDDR selected as the DAF is informed by the effects (clinical signs, tissue effects, biochemical changes) observed in the animal toxicity study and the aerosol characteristics in the inhalation study. The DAF is then applied to the duration-adjusted POD to arrive at the HEC of the POD (POD_{HEC}). The RDDR model was used herein to calculate HEC values from the aerosol exposures to animals available for each of the surfactant classes.

After an analogue(s) is identified, the strengths, limitations, and uncertainties associated with the use of the substance(s) to predict the hazards for the new chemical substance are considered when deriving a benchmark margin of exposure (MOE). The benchmark MOE is the result of multiplying all relevant UFs to account for: (1) the variation in susceptibility among the members of the human population (*i.e.*, inter- individual or intraspecies variability); (2) the extrapolation from animal data to humans (*i.e.*, interspecies extrapolation); (3) the extrapolation from data in a study with less- than- lifetime exposure (*i.e.*, extrapolating from sub-chronic to chronic exposure); (4) the extrapolation from a LOAEL to a NOAEL [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2002</Year><RecNum>14743</RecNum><DisplayText>[17, 19]</DisplayText><record><rec-number>14743</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019884">14743</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>A Review of the Reference Dose and Reference Concentration Processes</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>192, https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf</pages><volume>EPA/630/P-02/002F</volume><dates><year>2002</year></dates><urls></urls></record></Cite><Cite><Author>EPA</Author><Year>2014</Year><RecNum>14742</RecNum><record><rec-number>14742</rec-number><foreign-keys><key app="EN" db-

id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019768">14742</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation</title><secondary-title>Office of the Science Advisor, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of the Science Advisor, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>109, https://www.epa.gov/sites/production/files/2015-01/documents/ddef-final.pdf</pages><volume>EPA/R-14/002F</volume><dates><year>2014</year></dates><urls></urls></record></Cite></EndNote>]. EPA prefers using existing information to develop data-derived extrapolation factors (DDEFs) or chemical specific adjustment factors (CSAFs) rather than relying on default values [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2014</Year><RecNum>14742</RecNum><DisplayText>[19]</DisplayText><record><rec-number>14742</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019768">14742</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation</title><secondary-title>Office of the Science Advisor, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C.

[PAGE]

20460</secondary-title></titles><periodical><full-title>Office of the Science Advisor, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>109, https://www.epa.gov/sites/production/files/2015-01/documents/ddef-final.pdf</pages><volume>EPA/R-14/002F</volume><dates><year>2014</year></dates><urls></urls></record></Cite></EndNote>]. This investigation includes several approaches to derive DDEFs to use in assessing new surfactant chemical substances.

Exposure Assessment

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In assessing new chemical substances, generally new chemical substances do not have occupational exposure monitoring data; therefore, EPA typically develops exposure estimates for workers using the Chemical Screening Tool for Exposures and Environmental Releases (ChemSTEER) model. ChemSTEER estimates exposure as daily acute potential dose rates (PDRs) or lifetime average daily doses (LADDs). The PDR represents average exposure over an 8-hour workday, whereas the LADD estimates long-term exposures to the chemical substance, and is averaged over a lifetime exposure of 75 years. The PDR, an initial conservative exposure estimate, is the more appropriate dose-metric for estimating risks to surfactants because surfactants are surface-active at the point of exposure and lung effects occur rapidly following exposure. For chemical substances used in a liquid, mist, or aerosol form, the general default PDR values are 1.875 mg/kg-bw/day for inhalable aerosols or 0.625 mg/kg-bw/day for respirable aerosols as shown in [REF _Ref46930162 \h * MERGEFORMAT] [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2015</Year><RecNum>14745</RecNum><DisplayText>[20]</DisplayText><record><rec-number>14745</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr"

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timestamp="1596021217">14745</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>ChemSTEER User Guide, Chemical Screening Tool for Exposures and Environmental Releases</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>403, https://www.epa.gov/sites/production/files/2015-05/documents/user_guide.pdf</pages><dates><year>2015</year></dates><urls></urls></record></Cite></EndNote>].

Table [SEQ Table * ARABIC]. Default values used for calculating the daily acute potential dose rate (PDR).

Description	Equation	Description	Equation[a]	Defaults	Units
PDR (mg/kg-bw/day)	I/BW	Inhalation PDR (I)	Cm × b × h, where Cm is the mass concentration of chemical in air, b is the volumetric inhalation rate ($0 < b \leq 7.9$), and h is the exposure duration ($0 \leq h \leq 24$)	Cm = 15 mg/m^3 b = 1.25 m^3/hr h = 8 hours/day	mg/day
		Body weight (BW)	BW ($0 \leq BW$)	80 kg-bw	kg-bw

[a] Cm may also be adjusted for the mass concentration of the chemical with a permissible exposure limit (PEL) in air (based on the U.S. Occupational Safety and Health Administration [OSHA] PEL – time-weighted average [TWA]; where: KCk = the mass concentration limit of total particulate in air (mg/m^3) with a default of 15 mg/m^3 for inhalable and 5 mg/m^3 for respirable, Ys= the weight fraction of chemical in particulate ($0 < Ys \leq 1$), Ypel=the weight fraction of chemical or metal in particulate with a known PEL ($0 < Ypel \leq 1$) using the following equation: Cm = KCk × Ys/Ypel

The PDR is calculated using an exposure regimen for a default worker of 8 hours/day and 5 days/week, unless chemical-specific manufacture, processing or use information are provided in the PMN. The exposure conditions in animal studies often do not reflect occupational exposure scenarios; therefore, a duration adjustment and a DAF (*i.e.,* RDDR value) are applied to the POD to derive HECs for exposed human populations according to Agency methods [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><DisplayText>[18]</DisplayText><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. This adjustment would optimally be made using physiologically-based pharmacokinetic modeling [ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><DisplayText>[18]</DisplayText><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>], but the data required to conduct such modelling rarely exist for new chemical substances. Therefore, occupational exposures are adjusted using particle deposition models with human ventilation rates during exertion (work) and exposure durations appropriate to the particular occupational setting and chemical use scenario.

Risk Characterization

Risk characterization is the final, integrative step of risk assessment. EPA's Risk Characterization Policy defines risk characterization as the integration of information from the hazard and exposure components of the risk assessment into an overall conclusion about risk that

is complete, informative, and useful for decision-making. The risk characterization conveys the risk assessor's judgment as to the nature and existence of (or lack of) human health or ecological risks [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2000</Year><RecNum>14747</RecNum><DisplayText>[21]</DisplayText><record><rec-number>14747</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021806">14747</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Risk Characterization</title><secondary-title>Office of Science Policy, Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Science Policy, Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>189, https://nepis.epa.gov/Exe/ZyPDF.cgi/40000006.PDF?Dockey=40000006.PDF</pages><volume>EPA 100-B-00-002</volume><dates><year>2000</year></dates><urls></urls></record></Cite></EndNote>]. As described in EPA's Risk Characterization Handbook "Risk characterization at EPA assumes different levels of complexity depending on the nature of the risk assessment being characterized and the level of information contained in each risk characterization varies according to the type of assessment for which the characterization is written and the audience for which the characterization is intended."

Under TSCA section 5, EPA must determine whether a chemical substance presents an unreasonable risk of injury to health or the environment under the conditions of use. EPA generally uses an MOE approach to characterize risks of new chemical substances as a starting point to estimate non-cancer risks for acute and chronic exposures. The MOE is the HEC derived from a POD for a health endpoint (from hazard assessment) divided by the exposure concentration for the scenario of concern (from exposure assessment). The calculated MOE is compared with a benchmark MOE to evaluate whether there is an adequate margin between human exposure estimates and the HEC . When the MOE is less than the benchmark MOE, there is a possibility of human health risks. On the other hand, negligible concerns would be expected if the MOE exceeds the benchmark MOE. The MOE approach is a widely recognized point estimate method and provides a risk profile for different non-cancer health effects and different exposure scenarios.

In summary, in developing a risk assessment for new chemical substances under TSCA section 5, EPA uses empirical data or analogues, to identify a POD(s) and to develop an exposure estimate for use in the evaluation. The hazard assessment in combination with the exposure assessment is used to calculate an MOE, which is compared to the benchmark MOE to identify potential risks. The risk characterization is used to inform the TSCA "unreasonable risk" determination.

RESULTS AND DISCUSSION

Literature Search and Screening Results

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Need to re-write

An initial search of PubMed identified 594 articles that were subjected to title and abstract screening. Of these articles, 551 did not meet the PECO criteria, whereas 43 met the PECO criteria and were selected for full text review. An additional 17 articles that met the PECO criteria were identified through additional search strategies, screening gray literature, references for other types of chemical substances, *etc*., and were included for full text review. Of the 60 articles evaluated through full text screening, 25 were identified as relevant and carried forward in the present evaluation, whereas the remaining 35 articles were excluded because they lacked relevant information on respiratory tract effects or presented inconclusive epidemiology findings. In the supplemental literature search of PubMed and Embase, 1247 articles (combined) were identified. Following title and abstract screening, 1217 of these articles were excluded because they did not meet the PECO criteria, whereas 25 met the PECO criteria and were selected for full text review. An additional 10 studies that met the PECO criteria were found by additional hand searching), and were selected for full text screening, which resulted in 35 articles that were identified for review; ten articles were deemed irrelevant and excluded. A total of 25 articles were identified in both of the searches, one was excluded because it was in a foreign language and of the remaining 24 articles are summarized in Table 8 in the Supporting Information file at "Section 1 Systematic Literature Review".

~~The initial PubMed search identified 594 articles that were subjected to title and abstract screening. Of these articles, 551 did not meet the PECO criteria, whereas 43 met the PECO criteria and were selected for full text review. An additional 17 articles were included for full text review that met the PECO criteria and were identified through additional search strategies, screening gray literature, references for other types of chemical substances, *etc*., including 9 additional studies found during the supplemental literature search described below. Of the 60~~

Commented [HT10]: Search 1 = 43 + 8 = 51 to full text review 51 -

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Without the 9 from supplemental was it 8 that were identified from additional strategies?

articles evaluated through full text screening. 25 were identified as relevant and carried forward in the present evaluation, whereas the remaining 35 articles were excluded because they lacked relevant information on respiratory tract effects or presented inconclusive epidemiology findings. In the supplemental literature search, 1247 articles were identified on PubMed and Embase (combined). Following title and abstract screening, 1212 of these articles were excluded because they did not meet the PECO criteria. A total of 35 articles (including 10 studies found by additional hand searching) met the PECO criteria and were selected for full text screening, which resulted in 25 articles that were identified for review; ten articles were deemed irrelevant and excluded. Of the 25 articles identified for review, 9 of the studies were additional studies from the supplemental literature search.

Commented [HT12]: Search 2 = 35
35 – 10 irrelevant = 25

Commented [HT13]: Isn't this the SAME 25 and 10 as above – highlighted???

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The information identified in the systematic review was used to determine Category Boundaries and subcategories, to summarize the health effects of surfactants under the section on Hazard Identification, and to identify potential NAMs for use in the Tiered-Testing Strategies.

Category Boundaries

The following structural and functional criteria (hereinafter referred to as the "Surfactant Criteria") are used to distinguish chemical substances, which include polymers and UVCB substances,[2] intended for use as surfactants from other amphiphilic compounds (*e.g.*, ethanol) [ADDIN EN.CITE ADDIN EN.CITE.DATA]:

[2] Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials (UVCB Substance)

1. A substance which has surface-active properties, and which consists of one or more hydrophilic and one or more hydrophobic groups;
2. The substance is capable of reducing the surface tension between air and water to 45 milliNewtons/meter (mN/m) or below at a test condition of 0.5 wt% in water and a temperature of 20°C (*Cf.* Pure water has a surface tension of 72.8 mN/m at 20°C); and
3. The substance self-associates in water to form micellar or vesicular aggregates at a concentration of 0.5 wt% or less.

Commented [HT15]: Condition or Concentration???

Criteria #3 says Concentration

The Surfactants Category is further defined into three general subcategories including nonionic, anionic, and cationic substances. Amphoteric chemical substances that meet the Surfactant Criteria would also be included within these subcategories (*i.e.*, anionic and cationic surfactants), depending on their pH. Lung lining fluids are near neutral pH, with various measurements ranging from 6.6 to 7.1 [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The pKa for each component of an amphoteric surfactant should be evaluated within this pH range and the assessment should be conducted on the predominant components. The non-ionized fraction for acids/bases is calculated as follows:

Commented [HT16]: In Table 3 footnote, called Zwitterionic I vote to change to Amphoteric in Table 3

$$\text{Acids Fraction}_{\text{non-ionized}} = 1 / (1 + 10^{\text{pH-pKa}})$$

$$\text{Bases Fraction}_{\text{non-ionized}} = 1 / (1 + 10^{\text{pKa-pH}})$$

Where the pH represents the physiological pH in the lung lining fluid (*i.e.*, 6.6 to 7.1), and the pKa represents the value for the respective component (*e.g.*, carboxylic acid or amine).

Nonionic surfactants are identified as any neutral chemical substance that meets the Surfactant Criteria. Common nonionic surfactants include alkylphenol chemical substances with one or more ethoxylate (EO) unit as well as linear and branched alcohol chemical substances with one or more EO units. For example, octylphenoxypolyethoxyethanol, a common nonionic octylphenol EO surfactant, and Polysorbate 80 (or Tween 80), another nonionic alkyphenol ethoxylate with increased alkyl chain length and number of EO units, are shown in [REF _Ref47613375 \h * MERGEFORMAT]. The surface tensions of octylphenoxypolyethoxyethanol and Polysorbate 80 range from 30-31 mN/m to 37.96 mN/m, respectively ([REF _Ref47613375 \h * MERGEFORMAT]) [ADDIN EN.CITE <EndNote><Cite><Author>Kothekar</Author><Year>2007</Year><RecNum>14758</RecNum><DisplayText>[28]</DisplayText><record><rec-number>14758</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596025228">14758</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kothekar, S.C.</author><author>Ware, A.M.</author><author>Waghmare, J.T.</author><author>Momin, S.A.</author></authors></contributors><titles><title>Comparative Analysis of the Properties of Tween-20, Tween-60, Tween-80, Arlacel-60, and Arlacel-80</title><secondary-title>Journal of Dispersion Science and Technology</secondary-title></titles><periodical><full-title>Journal of Dispersion Science and Technology</full-title></periodical><pages>477-484, https://www.tandfonline.com/doi/abs/10.1080/01932690601108045</pages><volume>28</volu

me><number>3</number><dates><year>2007</year></dates><urls></urls></record></Cite></EndNote>].

Anionic surfactants are identified as any chemical substance with a net negative charge that meets the Surfactant Criteria (*e.g.*, alkyl sulfonates, alkylbenzene sulfonates, alkylether sulfates, alkyl silicic acids, alkyl phosphates, alkyl carboxylic acids, or combinations of these anionic groups). An example anionic surfactant, SDS, has a reported surface tension of 35 mN/m ([REF _Ref47613375 \h * MERGEFORMAT]).

Cationic surfactants are identified as any chemical substance with a net positive charge that meets the Surfactant Criteria (*e.g.*, alkylammonium chlorides and benzalkonium chlorides). Benzalkonium chloride (BAC: CASRN 8001-54-5) and DDAC are representative members of this subcategory, with surface tensions of 37 mN/m and 25.82 mN/m ([REF _Ref47613375 \h * MERGEFORMAT]), respectively. It is noted that BAC and DDAC also possess biocidal properties.

Typical commercial surfactants (nonionic, anionic, and cationic) are non-volatile liquids or solids. This category framework focuses on exposure *via* aerosol forms (*i.e.*, both airborne droplets and solid particles, including the hygroscopic variety) of these surfactants. While the commercial use of volatile surfactants is unlikely, it should be noted that this framework is not applicable to any substances that qualify as surfactants and are volatile under the conditions of use.

Table [SEQ Table * ARABIC]. Example Chemicals that Meet "Surfactant Criteria" and Nonionic, Anionic and Cationic Subcategorization.

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Nonionic Surfactants					
Chemical Name in Text	**Other Relevant Names**	**Criteria 1**		**Criteria 2**	**Criteria 3**
		Hydrophobic group(s)	**Hydrophilic group(s)**	**Surface Tension**	**Critical Micelle Concentration (CMC)**
formaldehyde, polymer with oxirane and 4-(1,1,3,3-tetramethylbutyl)-phenol Defomaire Alevaire Tyloxapol CASRN: 25301-02-4	CAS Name: formaldehyde, polymer with oxirane and 4-(1,1,3,3-tetramethylbutyl)-phenol	multiple octyl phenol groups	multiple polyoxyethylene (9) units	~37 mN/m at 5 g/L (0.5 wt%) and 25°C* [ADDIN EN.CITE <EndNote><Cite><Author>Schott</Author><Year>1998</Year><RecNum>14754</RecNum><DisplayText>[29]</DisplayText><record><rec-number>14754</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596024000">14754</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Schott,	0.038 g/L or 0.0038 wt% [ADDIN EN.CITE <EndNote><Cite><Author>Schott</Author><Year>1998</Year><RecNum>14754</RecNum><DisplayText>[29]</DisplayText><record><rec-number>14754</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596024000">14754</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sch

				H.</author></authors></contributors><auth-address>School of Pharmacy, Temple University, Philadelphia, Pennsylvania, 19140</auth-address><titles><title>Comparing the Surface Chemical Properties and the Effect of Salts on the Cloud Point of a Conventional Nonionic Surfactant, Octoxynol 9 (Triton X-100), and of Its Oligomer, Tyloxapol (Triton WR-1339)</title><secondary-title>J Colloid Interface Sci</secondary-title><alt-title>Journal of colloid and interface science</alt-title></titles><periodical><full-title>Journal of colloid and interface science</full-title><abbr-1>J Colloid Interface Sci</abbr-	ott, H.</author></authors></contributors><auth-address>School of Pharmacy, Temple University, Philadelphia, Pennsylvania, 19140</auth-address><titles><title>Comparing the Surface Chemical Properties and the Effect of Salts on the Cloud Point of a Conventional Nonionic Surfactant, Octoxynol 9 (Triton X-100), and of Its Oligomer, Tyloxapol (Triton WR-1339)</title><secondary-title>J Colloid Interface Sci</secondary-title><alt-title>Journal of colloid and interface science</alt-title></titles><periodical><full-title>Journal of colloid and interface

				1></periodical><alt-periodical><full-title>Journal of colloid and interface science</full-title><abbr-1>J Colloid Interface Sci</abbr-1></alt-periodical><pages>496-502</pages><volume>205</volume><number>2</number><edition>1998/12/16</edition><dates><year>1998</year><pub-dates><date>Sep 15</date></pub-dates></dates><isbn>0021-9797</isbn><accession-num>9735215</accession-num><urls></urls><electronic-resource-num>10.1006/jcis.1998.5721</electronic-resource-num><remote-database-provider>NLM</remote-database-	science</full-title><abbr-1>J Colloid Interface Sci</abbr-1></alt-periodical><full-title>Journal of colloid and interface science</full-title><abbr-1>J Colloid Interface Sci</abbr-1></alt-periodical><pages>496-502</pages><volume>205</volume><number>2</number><edition>1998/12/16</edition><dates><year>1998</year><pub-dates><date>Sep 15</date></pub-dates></dates><isbn>0021-9797</isbn><accession-num>9735215</accession-num><urls></urls><electronic-resource-num>10.1006/jcis.1998.5721</electronic-resource-

				provider><language>eng</language></record></Cite></EndNote>]	num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]
octylphenoxypolyethoxyethanol CASRN: 9002-93-1	Triton X-100 Octoxynol 9 octylphenol ethoxylate CAS Name: poly(oxy-1,2-ethanediyl), .alpha.-[4-1,1,3,3-tetramethylbutyl)phenyl]-.omega.-hydroxy	octylphenol group	polyoxyethylene (9) unit	~30.5 mN/m at 5 g/L (0.5 wt%) and 25°C* [ADDIN EN.CITE <EndNote><Cite><Author>Schott</Author><Year>1998</Year><RecNum>14754</RecNum><DisplayText>[29]</DisplayText><record><rec-number>14754</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596024000">14754</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Schott, H.</author></authors>	0.17 g/L or 0.017 wt% [ADDIN EN.CITE <EndNote><Cite><Author>Schott</Author><Year>1998</Year><RecNum>14754</RecNum><DisplayText>[29]</DisplayText><record><rec-number>14754</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596024000">14754</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Schott,

				</contributors><auth-address>School of Pharmacy, Temple University, Philadelphia, Pennsylvania, 19140</auth-address><titles><title>Comparing the Surface Chemical Properties and the Effect of Salts on the Cloud Point of a Conventional Nonionic Surfactant, Octoxynol 9 (Triton X-100), and of Its Oligomer, Tyloxapol (Triton WR-1339)</title><secondary-title>J Colloid Interface Sci</secondary-title><alt-title>Journal of colloid and interface science</alt-title></titles><periodical><full-title>Journal of colloid and interface science</full-title><abbr-1>J Colloid Interface Sci</abbr-1></periodical><alt-	H.</author></authors></contributors><auth-address>School of Pharmacy, Temple University, Philadelphia, Pennsylvania, 19140</auth-address><titles><title>Comparing the Surface Chemical Properties and the Effect of Salts on the Cloud Point of a Conventional Nonionic Surfactant, Octoxynol 9 (Triton X-100), and of Its Oligomer, Tyloxapol (Triton WR-1339)</title><secondary-title>J Colloid Interface Sci</secondary-title><alt-title>Journal of colloid and interface science</alt-title></titles><periodical><full-title>Journal of colloid and interface science</full-

				periodical><full-title>Journal of colloid and interface science</full-title><abbr-1>J Colloid Interface Sci</abbr-1></alt-periodical><pages>496-502</pages><volume>205</volume><number>2</number><edition>1998/12/16</edition><dates><year>1998</year><pub-dates><date>Sep 15</date></pub-dates></dates><isbn>0021-9797</isbn><accession-num>9735215</accession-num><urls></urls><electronic-resource-num>10.1006/jcis.1998.5721</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>e	title><abbr-1>J Colloid Interface Sci</abbr-1></periodical><alt-periodical><full-title>Journal of colloid and interface science</full-title><abbr-1>J Colloid Interface Sci</abbr-1></alt-periodical><pages>496-502</pages><volume>205</volume><number>2</number><edition>1998/12/16</edition><dates><year>1998</year><pub-dates><date>Sep 15</date></pub-dates></dates><isbn>0021-9797</isbn><accession-num>9735215</accession-num><urls></urls><electronic-resource-num>10.1006/jcis.1998.5721</electronic-resource-num><remote-

				ng</language></record></Cite></EndNote>]	database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]
polyoxyethylene-10-oleyl ether ($C_{18:1}E_{10}$) CASRN: 9004-98-2	oleyl ethoxylate CAS Name: poly(oxy-1,2-ethanediyl), .alpha.-(9Z)-9-octadecen-1-yl-.omega.-hydroxy	oleyl group	polyoxyethylene (10) unit	35.17 mN/m at 4×10^{-5} M (0.028 wt%) and 25°C* [ADDIN EN.CITE <EndNote><Cite><Author>Liu</Author><Year>2006</Year><RecNum>14761</RecNum><DisplayText>[30]</DisplayText><record><rec-number>14761</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596025582">14761</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Liu,	4×10^{-5} M or 0.028 wt % at 25°C [ADDIN EN.CITE <EndNote><Cite><Author>Liu</Author><Year>2006</Year><RecNum>14761</RecNum><DisplayText>[30]</DisplayText><record><rec-number>14761</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596025582">14761</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Liu,

				F.</author><author>Wang, Z.</author><author>Sun, D.</author><author>Wei, X.</author><author>Zhou, W.</author><author>Li, G.</author><author>Zhang, G.</author></authors></contributors><titles><title>Adsorption Kinetics of Brij 97 at the Air/Solution Interface</title><secondary-title>Journal of Dispersion Science and Technology</secondary-title></titles><periodical><full-title>Journal of Dispersion Science and Technology</full-title></periodical><pages>657-663, https://www.tandfonline.com/doi/abs/10.1080/01932690600660624</pages><volume>27	F.</author><author>Wang, Z.</author><author>Sun, D.</author><author>Wei, X.</author><author>Zhou, W.</author><author>Li, G.</author><author>Zhang, G.</author></authors></contributors><titles><title>Adsorption Kinetics of Brij 97 at the Air/Solution Interface</title><secondary-title>Journal of Dispersion Science and Technology</secondary-title></titles><periodical><full-title>Journal of Dispersion Science and Technology</full-title></periodical><pages>657-663, https://www.tandfonline.com/doi/abs/10.10

				</volume><number>5</number><dates><year>2006</year></dates><urls></urls></record></Cite></EndNote>]	80/019326906006606 24</pages><volume> 27</volume><numbe r>5</number><dates ><year>2006</year> </dates><urls></urls ></record></Cite></ EndNote>]
polyoxyethylene-10-dodecyl ether ($C_{12}E_{10}$) CASRN: 9002-92-0	polyoxyethylene (10) lauryl ether CAS Name: poly(oxy-1,2-ethanediyl),-.alpha.-dodecyl-.omega.-	dodecyl group	polyoxyethylene (10) unit	C12E9: 36 mN/m (concentration not reported) at 23°C* C12E12: 32 mN/m (concentration not reported) at 23°C* [ADDIN EN.CITE <EndNote><Cite><Au thor>Rosen</Author> <Year>1989</Year>< RecNum>14763</Rec Num><DisplayText>[31]</DisplayText><re cord><rec-number>14763</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0 azr5evearxfds0err5sr" timestamp="15960265 43">14763</key></for eign-keys><ref-type name="Edited	12.7×10^{-6} M or 0.0008 wt% at 30°C [ADDIN EN.CITE <EndNote><Cite><A uthor>Sulthana</Aut hor><Year>2000</Y ear><RecNum>1476 2</RecNum><Displa yText>[32]</Display Text><record><rec-number>14762</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre 0azr5evearxfds0err5s r" timestamp="1596025 808">14762</key></ foreign-keys><ref-type name="Journal Article">17</ref-type><contributors>< authors><author>Sult hana,

				Book">28</ref-type><contributors><authors><author>Rosen, M.J.</author></authors></contributors><titles><title>Surfactants and interfacial phenomena</title></titles><pages>431, </pages><dates><year>1989</year></dates><pub-location>New York</pub-location><publisher>John Wiley & Sons, Inc.</publisher><urls></urls></record></Cite></EndNote>]	S.B.</author><author>Rao, P.V.C.</author><author>Bhat, S.G.T.</author><author>Sugihara, N.G.</author><author>Rakshit, A.K.</author></authors></contributors><titles><title>Solution Properties of Nonionic Surfactants and Their Mixtures: Polyoxyethylene (10) Alkyl Ether [CnE10] and MEGA-10</title><secondary-title>Langmuir</secondary-title></titles><periodical><full-title>Langmuir : the ACS journal of surfaces and colloids</full-title><abbr-1>Langmuir</abbr-1></periodical><pages>980-987, https://doi.org/10.1021/la990730o</pages>

					<volume>16</volume><number>3</number><dates><year>2000</year></dates><urls></urls></record></Cite></EndNote>] Also, C12E9 at 1×10^{-6} M at 23°C and C12E12 at 1.4×10^{-6} M at 23°C [ADDIN EN.CITE <EndNote><Cite><Author>Rosen</Author><Year>1989</Year><RecNum>14763</RecNum><DisplayText>[31]</DisplayText><record><rec-number>14763</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596026543">14763</key></foreign-keys><ref-type name="Edited Book">28</ref-type><contributors><authors><author>Rosen,

					M.J.</author></authors></contributors><titles><title>Surfactants and interfacial phenomena</title></titles><pages>431, </pages><dates><year>1989</year></dates><pub-location>New York</pub-location><publisher>John Wiley & Sons, Inc.</publisher><urls></urls></record></Cite></EndNote>]
Polysorbate 20 (Tween 20) CASRN: 9005-64-5	polyoxyethylene (20) sorbitan monolaurate CAS Name: sorbitan, monododecanoate, poly(oxy-1,2-ethanediyl) derivs.	dodecanoyl group	sorbitan polyoxyethylene (20) unit	38 mN/m at 8.04×10^{-5} M (0.001 wt%) and 21°C* [ADDIN EN.CITE <EndNote><Cite><Author>Kim</Author><Year>2001</Year><RecNum>14756</RecNum><DisplayText>[33]</DisplayText><record><rec-number>14756</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0	8.04×10^{-5} M or 0.001 wt% at 21°C [ADDIN EN.CITE <EndNote><Cite><Author>Kim</Author><Year>2001</Year><RecNum>14756</RecNum><DisplayText>[33]</DisplayText><record><rec-number>14756</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5s

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				r>31</number><dates><year>2001</year></dates><urls></urls></record></Cite></EndNote>]	>187-188</volume><number>31</number><dates><year>2001</year></dates><urls></urls></record></Cite></EndNote>]
Polysorbate 80 (Tween 80) CASRN: 9005-65-6	polyoxyethylene (20) sorbitan monooleate CAS Name: sorbitan, mono-(9Z)-9-octadecenoate, poly(oxy-1,2-ethanediyl) derivs.	octadecenoyl group	sorbitan polyoxyethylene (20) unit	37.96 mN/m at 5 g/L (0.5 wt%) and 30°C [ADDIN EN.CITE <EndNote><Cite><Author>Kothekar</Author><Year>2007</Year><RecNum>14758</RecNum><DisplayText>[28]</DisplayText><record><rec-number>14758</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596025228">14758</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kothekar, S.C.</author><author>Ware,	1.5×10^{-5} M or 0.002 wt% at 25°C [ADDIN EN.CITE <EndNote><Cite><Author>Mahmood</Author><Year>2013</Year><RecNum>14757</RecNum><DisplayText>[34]</DisplayText><record><rec-number>14757</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596024783">14757</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Mahmood, M.E.</author><autho

				A.M.</author><author>Waghmare, J.T.</author><author>Momin, S.A.</author></authors></contributors><titles><title>Comparative Analysis of the Properties of Tween-20, Tween-60, Tween-80, Arlacel-60, and Arlacel-80</title><secondary-title>Journal of Dispersion Science and Technology</secondary-title></titles><periodical><full-title>Journal of Dispersion Science and Technology</full-title></periodical><pages>477-484, https://www.tandfonline.com/doi/abs/10.1080/01932690601108045</pages><volume>28</volume><number>3</number><dates><year>2007</year></dates><urls></urls></reco	r>Al-Koofee, D.A.F.</author></authors></contributors><titles><title>Effect of Temperature Changes on Critical Micelle Concentration for Tween Series Surfactants</title><secondary-title>Global Journal of Science Frontier Research Chemistry</secondary-title></titles><periodical><full-title>Global Journal of Science Frontier Research Chemistry</full-title></periodical><pages>5, https://journalofscience.org/index.php/GJSFR/article/view/816/681</pages><volume>13(B)</volume><number>4</number><dates><year>2013</year></dates><urls></urls></record></Cite></EndNote>]

				rd></Cite></EndNote >]	
Poloxamer 188 CASRN: 691397-13-4	CAS Name: oxirane, 2-methyl-, polymer with oxirane, triblock	polyoxypropylene (27) unit	two polyoxyethylene (80) units	~42-44 mN/m at ~0.5 wt% and 36°C [ADDIN EN.CITE ADDIN EN.CITE.DATA]	4.8×10^{-4} M or 0.4 wt% at 37°C [ADDIN EN.CITE ADDIN EN.CITE.DATA]
N,N-dimethyl-dodecylamine-N-oxide ($C_{12}AO$)*** CASRN: 1643-20-5	lauryl dimethylamine oxide CAS Name:1-dodecanamine, N,N-dimethyl-, N-oxide	dodecyl group	amine oxide unit	34.1 mN/m at 1 g/L (0.1 wt%) and 20°C [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14772</RecNum><DisplayText>[37]</DisplayText><record><rec-number>14772</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596028055">14772</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></aut	1.7×10^{-3} M or 0.039 wt% [ADDIN EN.CITE <EndNote><Cite><Author>Hoffmann</Author><Year>1990</Year><RecNum>14764</RecNum><DisplayText>[38]</DisplayText><record><rec-number>14764</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596026736">14764</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hoffmann,

				hors></contributors><titles><title>Dodecyldimethylamine oxide, CASRN: 1643-20-5, EC number: 216-700-6, Surface Tension</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/registration-dossier/-/registered-dossier/10062/4/11</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]	H.</author></authors></contributors><titles><title>Correlation between surface and interfacial tensions with micellar structures and properties of surfactant solutions</title><secondary-title>Progress in Colloid & Polymer Science</secondary-title></titles><periodical><full-title>Progress in Colloid & Polymer Science</full-title></periodical><pages>16-28, https://link.springer.com/chapter/10.1007%2FBFb0116238</pages><volume>18</volume><dates><year>1990</year></dates><urls></urls></record></Cite></EndNote>] 1×10^{-5} M to 5.5×10^{-5} M or 0.0002 to 0.001

					wt% at 25°C [ADDIN EN.CITE <EndNote><Cite><Author>Mukerjee</Author><Year>1971</Year><RecNum>14765</RecNum><DisplayText>[39]</DisplayText><record><rec-number>14765</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596026897">14765</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Mukerjee, P.</author><author>Mysels, K.J.</author></authors></contributors><titles><title>Critical micelle concentrations of aqueous surfactant systems</title><seco

					ndary-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</secondary-title></titles><periodical><full-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</full-title></periodical><pages>242, https://nvlpubs.nist.gov/nistpubs/Legacy/NSRDS/nbsnsrds36.pdf</pages><dates><year>1971</year></dates><urls></urls></record></Cite></EndNote>]
Anionic Surfactants					
Chemical	**Other Relevant Names**	**Criteria 1**		**Criteria 2**	**Criteria 3**

Name in Text		Hydrophobic group(s)	Hydrophilic group(s)	Surface Tension	Critical Micelle Concentration (CMC)
sodium dodecyl sulfate (SDS) CASRN: 151-21-3	CAS Name: sulfuric acid monododecyl ester sodium salt (1:1)	dodecyl group	sulfate group	35 mN/m at 0.29 wt% and 20°C [ADDIN EN.CITE <EndNote><Cite><Author>Hernainz</Author><Year>2002</Year><RecNum>14768</RecNum><DisplayText>[40]</DisplayText><record><rec-number>14768</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596027363">14768</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hernainz, F.</author><author>Caro, A.</author></authors></contributors><titles><title>Variation of surface tension in	8.25×10^{-3} M or 0.24 wt% at 20°C [ADDIN EN.CITE <EndNote><Cite><Author>Mukerjee</Author><Year>1971</Year><RecNum>14765</RecNum><DisplayText>[39]</DisplayText><record><rec-number>14765</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596026897">14765</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Mukerjee, P.</author><author>Mysels, K.J.</author></authors></contributors><titles><title>Critical

[PAGE]

				aqueous solutions of sodium dodecyl sulfate in the flotation batch</title><secondary-title>Colloids and Surfaces A: Physicochemical and Engineering Aspects</secondary-title></titles><periodical><full-title>Colloids and Surfaces A: Physicochemical and Engineering Aspects</full-title></periodical><pages>19-24, https://www.sciencedirect.com/science/article/abs/pii/S0927775701005751</pages><volume>196</volume><number>1</number><dates><year>2002</year></dates><urls></urls></record></Cite></EndNote>]	micelle concentrations of aqueous surfactant systems</title><secondary-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</secondary-title></titles><periodical><full-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</full-title></periodical><pages>242, https://nvlpubs.nist.gov/nistpubs/Legacy/NSRDS/nbsnsrds36.pdf</pages><dates><year>1971</year></dates><urls></urls></record></Cite></EndNote>]

oleoyl sarcosine CASRN: 110-25-8	CAS Name: glycine, N-methyl-N-((9Z)-1-oxo-9-octadecen-1-y	oleyl group	carboxylic acid anion	31.91 mN/m at 0.1 wt% and 19.9°C** [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14767</RecNum><DisplayText>[41]</DisplayText><record><rec-number>14767</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596027202">14767</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sodium N-methyl-N-(1-oxo-9-octadecenyl)aminoacetate, CASRN 3624-77-9, EC number: 222-829-9, Surface Tension</title><secon	2.6×10^{-3} wt% and ~25°C ** (temperature not reported, assumed to be room temperature) [ADDIN EN.CITE <EndNote><Cite><Author>ChattemChemicals</Author><Year>2020</Year><RecNum>14769</RecNum><DisplayText>[42]</DisplayText><record><rec-number>14769</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596027596">14769</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>ChattemChemicals</author></authors></contributors><titles><title>Oleoyl Sarcosine, CASRN 110-25-

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sodium lauroyl sarcosinate CASRN: 137-16-6	CAS Name: glycine, N-methyl-N-(1-oxododecyl)-, sodium salt (1:1)	lauryl group	carboxylic acid anion	40.5 mN/m at 2 wt% and 20°C [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14770</RecNum><DisplayText>[43]</DisplayText><record><rec-number>14770</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0	8.0×10^{-2} wt% and ~25°C (temperature not reported, assumed to be room temperature) [ADDIN EN.CITE <EndNote><Cite><Author>ChattemChemicals</Author><Year>2020</Year><RecNum>14769</RecNum><DisplayText>[42]</DisplayText><record><rec-number>14769</rec-

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				rls></urls></record></Cite></EndNote>]	ord></Cite></EndNote>]
dioctyl sulfosuccinate sodium salt (DOSS) CASRN: 577-11-7	dioctyl sodium sulfosuccinate CAS Name: Butanedioic acid, 2-sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt	two 2-ethyl hexyl groups	sulfosuccinate group	<28 mN/m at 0.5 vol% and 25°C* [ADDIN EN.CITE <EndNote><Cite><Author>Williams</Author><Year>1957</Year><RecNum>14755</RecNum><DisplayText>[44]</DisplayText><record><rec-number>14755</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596024180">14755</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Williams, E.F.</author><author>Woodberry, N.T.</author><author>Dixon, J.K.</author></authors></contributors><titles><title>Purification	6.8×10^{-4} M or 0.03 wt% at 25°C [ADDIN EN.CITE <EndNote><Cite><Author>Mukerjee</Author><Year>1971</Year><RecNum>14765</RecNum><DisplayText>[39]</DisplayText><record><rec-number>14765</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596026897">14765</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Mukerjee, P.</author><author>Mysels, K.J.</author></authors></contributors><titles><title>Critical micelle

				and surface tension properties of alkyl sodium sulfosuccinates</title><secondary-title>Journal of Colloid Science</secondary-title></titles><periodical><full-title>Journal of Colloid Science</full-title></periodical><pages>452-459</pages><volume>12</volume><number>5</number><dates><year>1957</year></dates><urls></urls></record></Cite></EndNote>]	concentrations of aqueous surfactant systems</title><secondary-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</secondary-title></titles><periodical><full-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</full-title></periodical><pages>242, https://nvlpubs.nist.gov/nistpubs/Legacy/NSRDS/nbsnsrds36.pdf</pages><dates><year>1971</year></dates><urls></urls></record></Cite></EndNote>]

Cationic Surfactants					
Chemical Name in Text	Other Relevant Names	Criteria 1		Criteria 2	Criteria 3
		Hydrophobic group(s)	Hydrophilic group(s)	Surface Tension	Critical Micelle Concentration (CMC)
benzalkonium chloride (BAC) CASRN: 8001-54-5	CAS Name: quaternary ammonium compounds, alkylbenzyldimethyl, chlorides	alkyl chains are C12, C14, C16 and C18 and benzyl group	quaternary nitrogen	37 mN/m at concentrations greater than about 4×10^{-4}M and 25°C* [ADDIN EN.CITE <EndNote><Cite><Author>Nandni</Author><Year>2013</Year><RecNum>14766</RecNum><DisplayText>[45]</DisplayText><record><rec-number>14766</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596027033">14766</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Nandni,	C12: reported values range from 2.3 - 8.5×10^{-3} M or 0.078 - 0.29 wt% at 25°C C14: 3.7×10^{-4} M or 0.014 wt% and ~25°C (temperature not stated; assumed to be room temperature) C16: 4.2×10^{-5} M or 0.0016 wt% at 23°C C18: reported values range from 7.1 - 8.5×10^{-6} M or 0.0003 - 0.00036 wt% at 23°C [ADDIN EN.CITE <EndNote><Cite><Author>Mukerjee</Author><Year>1971</Year><RecNum>14765</RecNum><DisplayText>[39]</Display

[PAGE]

				D.</author><author>Mahajan, R.K.</author></authors></contributors><titles><title>Micellar and Interfacial Behavior of Cationic Benzalkonium Chloride and Nonionic Polyoxyethylene Alkyl Ether Based Mixed Surfactant Systems</title><secondary-title>Journal of Surfactants and Detergents</secondary-title></titles><periodical><full-title>Journal of Surfactants and Detergents</full-title></periodical><pages>587-599, https://doi.org/10.1007/s11743-012-1427-z</pages><volume>16</volume><number>4</number><dates><year>2013</year></dates><urls></urls></record></Cite></EndNote>]	Text><record><rec-number>14765</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596026897">14765</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Mukerjee, P.</author><author>Mysels, K.J.</author></authors></contributors><titles><title>Critical micelle concentrations of aqueous surfactant systems</title><secondary-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC

					20234</secondary-title></titles><periodical><full-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</full-title></periodical><pages>242, https://nvlpubs.nist.gov/nistpubs/Legacy/NSRDS/nbsnsrds36.pdf</pages><dates><year>1971</year></dates><urls></urls></record></Cite></EndNote>]
didecyldimethyl ammonium chloride (DDAC) CASRN: 7173-51-5	CAS Name: 1-decanaminium, N-decyl-N,N-dimethyl-, chloride (1:1)	decyl groups	quaternary nitrogen	25.82 mN/m at 1 g/L (0.1 wt%) and 20°C [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14771</RecNum><DisplayText>[46]</DisplayText><record><rec-number>14771</rec-number><foreign-	0.39 g/L or 0.039 wt% at 25°C [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14771</RecNum><DisplayText>[46]</DisplayText><record><rec-number>14771</rec-number><foreign-

				keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596027946">14771</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Didecyldimethylammonium chloride, CASRN: 7173-51-5, EC number: 230-525-2, Surface Tension</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/registration-dossier/-/registered-	keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596027946">14771</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Didecyldimethylammonium chloride, CASRN: 7173-51-5, EC number: 230-525-2, Surface Tension</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/registration-

				dossier/5864/4/11</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]	dossier/-/registered-dossier/5864/4/11</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]

*Not all of the surface tension measurement references identified are run at exactly 20°C, but they are sufficiently close (within 5°C) so as not to affect the measurement. In addition, several measurements were run at 0.1% instead of the recommended 0.5%. Increasing the concentration to 0.5% is likely to lower the surface tension.

**Carboxylic acid compounds, such as oleoyl sarcosine, have a carboxyl group pKa value of ~5, thus at physiological pH values maintained near 7 in the lung, the carboxyl group will be 99% in the anionic form according the Henderson-Hasselbalch equation. Since sodium is the major cation in mammalian body fluids (~145 mM), the use of the sodium oleoyl sarcosine surface tension value is appropriate for its characterization.

***Zwitterionic: At pH 7, 90% expected to be nonionic; only small amount cationic.

Commented [HT18]: This is called Amphoteric in the general category explanation above

Hazard Identification

There is concern for dysfunction of mucus, epithelial lining fluid, and natural surfactant lining in the various regions of the respiratory tract from inhalation of surfactants. There is also evidence that some surfactants or similar structures may also interfere with the cell membrane of the epithelium in these same regions [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The capacity of exogenous surfactants to interfere with pulmonary surfactant and impair pulmonary function has been demonstrated in both human volunteers and in laboratory animals [51, 5-7]. The respiratory tract responses to inhaled surfactant aerosol is thought to be in proportion to the exposure concentration and duration, but available data on acute and repeated-dose effect levels are limited within each subcategory, which limits establishing a correlation between chemical properties and toxicity due to exposure methods (*e.g.,* generated aerosol droplet size).

Nonionic Surfactants

In vivo studies

Several studies were identified for the nonionic siliconized superinone respiratory detergent, formaldehyde, polymer with oxirane and 4-(1,1,3,3-tetramethylbutyl)phenol polymer (CASRN 25301-02-4; commonly known as Defomarie, Alevaire, and Tyloxapol). Healthy human volunteers demonstrated significantly decreased respiratory compliance following acute inhalation of Defomaire [ADDIN EN.CITE <EndNote><Cite><Author>Obenour</Author><Year>1963</Year><RecNum>13656</RecNum><DisplayText>[49]</DisplayText><record><rec-number>13656</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr"

timestamp="1479320595">13656</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Obenour, R. A.</author><author>Saltzman, H. A.</author><author>Sieker, H. O.</author><author>Green, J. L.</author></authors></contributors><titles><title>Effects of surface-active aerosols and pulmonary congestion on lung compliance and resistance</title><secondary-title>Circulation</secondary-title><alt-title>Circulation</alt-title></titles><periodical><full-title>Circulation</full-title><abbr-1>Circulation</abbr-1></periodical><alt-periodical><full-title>Circulation</full-title><abbr-1>Circulation</abbr-1></alt-periodical><pages>888-92</pages><volume>28</volume><edition>OBENOUR, R A
SALTZMAN, H A
SIEKER, H O
GREEN, J L
1963/11/01</edition><keywords><keyword>Aerosols</keyword><keyword>Alcohols</keyword><keyword>Ethanol</keyword><keyword>Heart Failure</keyword><keyword>Humans</keyword><keyword>Infusions, Parenteral</keyword><keyword>Injections, Intravenous</keyword><keyword>Lung</keyword><keyword>Lung Compliance</keyword><keyword>Pulmonary Edema</keyword><keyword>Respiratory Function Tests</keyword><keyword>Silicones</keyword><keyword>Sodium Chloride</keyword><keyword>Surface-Active Agents</keyword></keywords><dates><year>1963</year><pub-dates><date>Nov</date></pub-dates></dates><isbn>0009-7322 (Print)
0009-7322 (Linking)</isbn><accession-num>14079193</accession-num><call-num>0 (Aerosols)
0 (Alcohols)
0 (Silicones)
0 (Surface-Active Agents)
3K9958V90M (Ethanol)
451W47IQ8X (Sodium Chloride)</call-num><urls></urls><remote-database-

provider>NLM</remote-database-provider><language>Eng</language></record></Cite></EndNote>]. An increased minimum surface tension due to detergent was shown to be dose-dependent, using pulmonary surfactant extracted from dogs with the nonionic surfactant tyloxapol (Alevaire) *in vitro* [ADDIN EN.CITE ADDIN EN.CITE.DATA]. However, *in vivo* exposure of dogs to Alevaire (8 hour aerosol exposure; vehicle and concentration not reported) produced little effect (only 1/10 dogs exposed to Alevaire showed increased minimum surface tension). The results did not support the dose-dependence of the effect and indicated that small amounts of detergent in the lungs may not detectably alter the surface tension-surface area relationship and that alteration of surface tension is unlikely to occur during reasonable use [ADDIN EN.CITE ADDIN EN.CITE.DATA].

Inhalation studies using dogs and/or sheep exposed to nonionic surfactant, tyloxapol, resulted in reduced oxygen content of arterial blood due to impaired gas exchange in the lung, increased pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, and grossly visible pulmonary edema and atelectasis (*i.e.*, collapsed alveoli) [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In the study by Modell *et al.* (1969) [ADDIN EN.CITE ADDIN EN.CITE.DATA], no gross pathology differences were seen in detergent-exposed versus control lungs of dogs, although some portions of both control and exposed lungs were heavy and discolored reddish-purple, which may have been caused by fluid accumulation from the liquid aerosol exposures and/or the use of hypotonic saline in the study (0.45% NaCl) since these effects were not observed in lungs treated with a less dense aerosol. Normal appearances were observed in the remaining areas of the lungs.

Commented [HT19]: ?? why calling this 'alcohol' here...the Surfactant Criteria are supposed to **distinguish** a surfactant from other amphiphilic chemicals like alcohols

In rodents, irritation and inflammatory effects in the entire respiratory tract have been observed with varying degrees of severity. Acute inhalation exposure *via* nose-only administration for 4 hours in Wistar Han rats to a concentration of 5.1 mg/L (5,100 mg/m^3) to ~~Polysorbate 20~~Sorbitan monolaurate, ethoxylated (~~Tween 20~~; CASRN 9005-64-5), a chemical not irritating to the skin or eyes [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14776</RecNum><DisplayText>[50]</DisplayText><record><rec-number>14776</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596030693">14776</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sorbitan monolaurate, ethoxylated, 1 - 6.5 moles ethoxylated, CASRN: 9005-64-5, EC number: 500-018-3, Skin irritation/corrosion</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/13525/7/4/2</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], with an MMAD of 2.2 µm and a GSD of 2, did not result in an increase in mortalities, clinical signs, or abnormalities in the gross pathology [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14777</RecNum><DisplayText>[51]</DisplayText><record><rec-number>14777</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596030813">14777</key></foreign-keys><ref-type name="Journal

Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sorbitan monolaurate, ethoxylated 1 - 6.5 moles ethoxylated, CASRN: 9005-64-5, EC number: 500-018-3, Acute Toxicity: Inhalation</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/13525/7/3/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. A respiratory irritation study was performed on a mixture containing octylphenoxypolyethoxyethanol (CASRN 9002-93-1) [ADDIN EN.CITE ADDIN EN.CITE.DATA], which can be severely irritating to the skin and eyes, in male Webster mice exposed for 3 hours to concentrations of 12, 22, 51, 118, and 134 mg/m^3 with 30-60 minutes recovery time (MMAD and GSD not provided). Signs of pulmonary irritation were observed in animals at the two highest concentrations as indicated by a decrease in respiratory frequency; this response was preceded by an increase in respiratory frequency at the highest three concentrations without an increase in gross lung abnormalities, pulmonary edema, or lung weight [ADDIN EN.CITE <EndNote><Cite><Author>Alarie</Author><Year>1992</Year><RecNum>14778</RecNum><DisplayText>[52]</DisplayText><record><rec-number>14778</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596035219">14778</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Alarie, Y.</author><author>Stock, M.F.</author></authors></contributors><titles><title>Respiratory Irritancy on a Mixture containing Polyethylene Glycol Mono(Octyl)Phenyl Eether CAS #9035-19-5</title><secondary-

title>ChemView - U.S. Environmental Protection Agency</secondary-title></titles><periodical><full-title>ChemView - U.S. Environmental Protection Agency</full-title></periodical><pages>37, https://chemview.epa.gov/chemview/proxy?filename=09022526800b76c9_86960000465_09-26-2011_8D_PHCS_Original%20-%2086960000465.pdf</pages><dates><year>1992</year></dates><urls></urls></record></Cite></EndNote>]. An acute inhalation exposure study in Syrian hamsters exposed to 3.0 mg/L of octylphenoxypolyethoxyethanol with varying exposure durations showed that lung deposition directly corresponded to mortality with an LD_{50} of 1300-2100 µg with an MMAD of 1.47 µm and a GSD of 1.84 [ADDIN EN.CITE <EndNote><Cite><Author>Damon</Author><Year>1982</Year><RecNum>13323</RecNum><DisplayText>[53]</DisplayText><record><rec-number>13323</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1479320592">13323</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Damon, E. G.</author><author>Halliwell, W. H.</author><author>Henderson, T. R.</author><author>Mokler, B. V.</author><author>Jones, R. K.</author></authors></contributors><titles><title>Acute toxicity of polyethylene glycol p-isooctylphenol ether in syrian hamsters exposed by inhalation or bronchopulmonary lavage</title><secondary-title>Toxicology and applied pharmacology</secondary-title><alt-title>Toxicol Appl Pharmacol</alt-title></titles><periodical><full-title>Toxicology and Applied Pharmacology</full-title><abbr-1>Toxicol. Appl. Pharmacol.</abbr-1></periodical><pages>53-61</pages><volume>63</volume><number>1</number><edition>Damon, E

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G
Halliwell, W H
Henderson, T R
Mokler, B V
Jones, R K
1982/03/30</edition><keywords><keyword>Animals</keyword><keyword>Cricetinae</keyword><keyword>Detergents/ toxicity</keyword><keyword>Dose-Response Relationship, Drug</keyword><keyword>Female</keyword><keyword>Lethal Dose 50</keyword><keyword>Lung/ drug effects/pathology</keyword><keyword>Male</keyword><keyword>Mesocricetus</keyword><keyword>Octoxynol</keyword><keyword>Polyethylene Glycols/administration & dosage/ toxicity</keyword><keyword>Surface-Active Agents/ toxicity</keyword><keyword>Therapeutic Irrigation</keyword></keywords><dates><year>1982</year><pub-dates><date>Mar 30</date></pub-dates></dates><isbn>0041-008X (Print)
0041-008X (Linking)</isbn><accession-num>7071873</accession-num><call-num>0 (Detergents)
0 (Surface-Active Agents)
30IQX730WE (Polyethylene Glycols)
9002-93-1 (Octoxynol)</call-num><urls></urls><remote-database-provider>NLM</remote-database-provider><language>Eng</language></record></Cite></EndNote>]. The authors concluded that the deaths in these animals were likely the result of severe laryngeal edema and ulcerative laryngitis while the lower airways in these animals were relatively free of serious pathologies. The authors hypothesized that these observed effects were due to large tracheobronchial deposition following the aerosol exposure and the mucociliary clearance of the chemical resulted in a large concentration on the laryngeal mucosa, though laryngeal deposition is typically a function of aerodynamics. In the only 2-week whole-body inhalation study for nonionic surfactants, male and female Sprague-Dawley rats were exposed to 5.3 and 10.3 mg/m^3 (5/sex/dose; MMAD 1.8 µm, GSD 1.8) octylphenoxypolyethoxyethanol for 6 hours/day, 5

days/week [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Slight to minimal subacute inflammation of the alveolar walls and hyperplasia of the alveolar/bronchiolar epithelium was reported, in addition to an increase in slight discoloration of the lungs, increased lung weight, and mucoid nasal discharge; a LOAEC of 5.3 mg/m^3 was identified.

Mechanistic studies

In vitro studies of surfactant on cell membranes have provided evidence of possible modes of action (MOAs). Warisnoicharoen *et al.* (2003) [ADDIN EN.CITE ADDIN EN.CITE.DATA] evaluated the cytotoxicity of the nonionic surfactants polyoxyethylene-10-oleyl ether ($C_{18:1}E_{10}$; CASRN 9004-98-2), polyoxyethylene-10-dodecyl ether ($C_{12}E_{10}$; CASRN 9002-92-0), and N,N-dimethyl-dodecylamine-N-oxide ($C_{12}AO$; CASRN 1643-20-5) on submerged cultured human bronchial epithelium cells (16-HBE14o-) *in vitro*, using the MTT cell viability assay by exposing the cells to 0.1mL of the serially diluted microemulsion for 30 minutes followed by a 60 minute incubation with a MTT solution (particle size not reported). All surfactants tested were cytotoxic at concentrations near or below their critical aggregation (micellular) concentrations (as determined by surface tension measurements), suggesting that toxicity was due to the disruption caused by the partitioning of monomeric surfactant into the cell membrane.

Lindenberg *et al.* (2019) [ADDIN EN.CITE <EndNote><Cite><Author>Lindenberg</Author><Year>2019</Year><RecNum>14779</RecNum><DisplayText>[55]</DisplayText><record><rec-number>14779</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr"

timestamp="1596035601">14779</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Lindenberg, F.</author><author>Sichel, F.</author><author>Lechevrel, M.</author><author>Respaud, R.</author><author>Saint-Lorant, G.</author></authors></contributors><titles><title>Evaluation of Lung Cell Toxicity of Surfactants for Inhalation Route</title><secondary-title>Journal of Toxicology and risk assessment</secondary-title></titles><periodical><full-title>Journal of Toxicology and risk assessment</full-title></periodical><pages>https://doi.org/10.23937/2572-4061.1510022</pages><volume>5</volume><number>1</number><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>] evaluated the cytotoxic activity of the three nonionic polymeric surfactants Polysorbate 20, Polysorbate 80 (Tween 80; CASRN 9005-65-6), and Poloxamer 188 (CASRN 691397-13-4), which are commonly used in formulations of nebulized pharmaceuticals to prevent protein agglomeration, in a BEAS-2B human bronchial epithelial cell model using an innovative air-liquid interface (ALI) method of exposure to surfactants with a nasal spray system (MMAD and GSD not provided). In this study, the ALI results were compared to the classical submerged cell culture or liquid/liquid (L/L) model. The study measured the release of Lactate Dehydrogenase (LDH), an intercellular enzyme present in the cytoplasm, indicative of the loss of membrane integrity. Cytotoxicity of Polysorbate 20 was observed at concentrations of 1-2% (v/v) when using the more biologically relevant ALI method; however, a significant increase in LDH was only observed at 4% for Polysorbate 80 and not significantly increased at concentrations of up to 10% for Poloxamer 188. These results suggest that Polysorbate 20 and to a lesser extent, Polysorbate 80 induce damage to the cell membrane integrity while the linear Poloxamer 188 did not demonstrate any *in vitro* cytotoxicity.

The available *in vitro* and *in vivo* data indicate inconsistency in respiratory toxicity among nonionic surfactants; however, the degree to which the variation is due to experimental design or bioactivity of the surfactant is not discernible from these data. The small dataset presented in this section preclude establishing correlations between respiratory effects and chemical properties, such as surface tension or CMC. Similarly, the examination of the relationship between chemical properties of nonionic surfactants and eye irritation has not established that hydrophilic-lipophilic balance, pH, alkyl chain length, or poly [oxyethylene] chain lengths can be used to predict eye irritation potential across the nonionic surfactant subcategory [ADDIN EN.CITE <EndNote><Cite><Author>Heinze</Author><Year>1999</Year><RecNum>14780</RecNum><DisplayText>[56]</DisplayText><record><rec-number>14780</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596035990">14780</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Heinze, J.E.</author><author>Casterton, P.L.</author><author>Atrash, J.</author></authors></contributors><titles><title>Relative Eye Irritation Potential of Nonionic Surfactants: Correlation to Dynamic Surface Tension</title><secondary-title>Journal of toxicology: cutaneous and ocular toxicology</secondary-title></titles><periodical><full-title>Journal of toxicology: cutaneous and ocular toxicology</full-title></periodical><pages>359-374, https://doi.org/10.3109/15569529909065552</pages><volume>18</volume><dates><year>1999</year></dates><urls></urls></record></Cite></EndNote>]. However, significant correlations of eye irritation and the maximum reduction in surface tension were observed at the CMC or

Commented [HT20]: Is discrepancy the correct word, or 'range'? or inconsistency?
Changed to inconsistency

higher surfactant concentration when surface tension was measured under dynamic conditions (0.24, 1, and 4 bubbles/second). Whether this chemical property similarly predicts potency of nonionic surfactants for respiratory effects requires additional data and analysis outside of the scope of this summary.

Anionic Surfactants

In vivo studies

Two acute inhalation toxicity studies were identified for anionic surfactants, which demonstrated high toxicity *via* the inhalation route. Oleoyl sarcosine (CASRN 110-25-8), irritating to the skin and damaging to the eye [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14781</RecNum><DisplayText>[57]</DisplayText><record><rec-number>14781</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596036160">14781</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>N-methyl-N-[C18-(unsaturated)alkanoyl]glycine, CASRN: NA, EC number: 701-177-3, Skin irritation/corrosion</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/21429/7/4/2/?documentUUID=fbaef057-ecc7-4763-aa56-1fa2c88c606c</pages><dates><year>2020</year></dates><urls></urls></record></Cite></End

Note>], was evaluated in a 4-hour nose-only inhalation study in male and female Sprague-Dawley rats at concentrations of 0.3, 0.6, 2.2, and 3.7 mg/L (300, 600, 2,200, 3,700 mg/m^3). The MMAD and GSD were not reported. An LC_{50} of 1.37 mg/L was identified with edema of the lung at 0.6 mg/L and audible gasping at 0.3 mg/L. For sodium lauroyl sarcosinate (CASRN 137-16-6), irritating to the skin and corrosive to the eye (undiluted), male Wistar rats were exposed to a 4-hour nose-only inhalation concentration of 0.05, 0.5, 1, and 5 mg/L (50, 500, 1,000, and 5,000 mg/m^3) with a MMAD 4.4, 2.9, 3.7, and 6.0 µm; GSD 2.7, 3, 4.2, and 2.9, respectively; 5 female rats were exposed to 1.1 or 5.5 mg/L with a MMAD 3.7 or 6.0 µm and GSD of 4.2 or 2.9, respectively [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14782</RecNum><DisplayText>[58, 59]</DisplayText><record><rec-number>14782</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596036284">14782</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sodium N-lauroylsarcosinate, CASRN: 137-16-6, EC number: 205-281-5, Eye Irritation</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/14123/7/4/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14783</RecNum><record><rec-number>14783</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596036540">14783</key></foreign-

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keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sodium N-lauroylsarcosinate, CASRN: 137-16-6, EC number: 205-281-5, Acute Toxicity: Inhalation</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/14123/7/3/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. The 5 mg/L dose resulted in fatality in all 10 animals tested within 1-2 h of dosing and the 0.5 mg/L dose resulted in fatality for 4/5 of the animals and exposure to 1 mg/L resulted in fatalities for the 10 animals within 1-2 days of exposure. Animals exposed to 0.05 mg/L did not demonstrate any adverse clinical signs or mortality at the conclusion of the study. At necropsy, red foci were noted on the lungs in animals receiving concentrations of ≥ 0.5 mg/L. The LC_{50} was reported to be 0.05-0.5 mg/L.

Repeated-dose inhalation studies were identified for oleoyl sarcosine, and dioctyl sodium sulfosuccinate (CASRN 577-11-7). Oleoyl sarcosine was evaluated in a 28-day nose-only inhalation study (6 hours/day, 5 days/week; Organization for Economic Cooperation and Development [OECD] Test Guideline 412) in male and female Fischer rats (5/group/sex) using concentrations of 0, 0.006, 0.02, or 0.06 mg/L (0, 6, 20, or 60 mg/m^3). The particle exposure MMAD was 1.11, 1.15, or 1.22 µm, GSD 1.68-2.57, and density 0.79 g/cm^2 for 6 hours/day, 5 days/week in 10% ethanol [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14784</RecNum

><DisplayText>[60]</DisplayText><record><rec-number>14784</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596036869">14784</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>N-methyl-N-[C18-(unsaturated)alkanoyl]glycine, CASRN: NA, EC number: 701-177-3, Repeated dose toxicity: Inhalation</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/21429/7/6/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. Changes in the mean corpuscular volume (MCV), white blood cells (WBC), and lymphocytes were observed in male animals at the high exposure concentration. In female animals of the mid-concentration exposure group, reticulocyte counts were significantly reduced. Reflex bradypnea was noted in the animals at the mid and high concentrations, which is associated with severely irritating substances. All test concentrations caused effects at several sites of the respiratory tract with indications for local irritation, such as squamous metaplasia and epithelium proliferation and submucous acute inflammation at the base of the epiglottis. In the alveoli walls and bronchi, the most prominent finding was a focal early stage of fibrosis, but details were not provided at the dose level for this effect. Lung weights were increased at the highest dose. The LOAEC was 0.006 mg/L (6 mg/m^3) air in males and females; the basis for the effect level was local irritation.

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Dioctyl sulfosuccinate sodium salt (DOSS; CASRN 577-11-7) was evaluated in a 13-week inhalation study in male and female Sprague-Dawley rats (12/group/sex), exposed to an aerosol of a product containing 0.0042 mg/L (4.2 mg/m^3) DOSS, for 4 hours a day, 5 days a week (as reported in a secondary source; MMAD and GSD not reported) [ADDIN EN.CITE <EndNote><Cite><Author>CIR</Author><Year>2013</Year><RecNum>14785</RecNum><DisplayText>[61]</DisplayText><record><rec-number>14785</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596037107">14785</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>CIR</author></authors></contributors><titles><title>Safety Assessment of Alkyl Sulfosuccinate Salts as Used in Cosmetics, Re-Review, CIR Expert Panel Meeting, June 10-11, 2013</title><secondary-title>Cosmetic Ingredient Review (CIR), Washington, D.C.</secondary-title></titles><periodical><full-title>Cosmetic Ingredient Review (CIR), Washington, D.C.</full-title></periodical><pages>171, https://www.cir-safety.org/sites/default/files/Sulfosuccinates_RR.pdf</pages><dates><year>2013</year></dates><urls></urls></record></Cite></EndNote>]. There were no statistically significant differences in exposed and control groups for the mean body weight gain, survival, appearance and behavior, urinalysis values, and microscopic lesions. Significant differences were noted in the blood as indicated by elevated erythrocytic values (not otherwise specified) at 7 weeks and depressed mean corpuscular hemoglobin concentration values at 13 weeks in male rats. In females, depressed serum glutamic pyruvic transaminase and significant effect on absolute heart weight was observed at 7 weeks, depressed serum alkaline phosphatase was observed at 13 weeks and elevated glucose at 7 and 13-weeks. At 7 weeks, the lungs of necropsied animals showed

scattered foci of neutrophils and an increase in alveolar macrophages were reported in a single exposed male rat. A LOAEC of 4.2 mg/m^3 was identified based on the blood effects in male rats.

Mechanistic studies

Mechanistic studies on the pulmonary effects of anionic surfactants have been studied in dogs and/or sheep exposed to DOSS.

Increased minimum surface tension of lung extract or bronchioalveolar lavage fluid (BALF) was observed in dogs and sheep following *in vivo* aerosol exposure to DOSS in 1:1 mixture of ethanol and saline for 30 – 60 minutes, at a concentration that was selected to ensure a moderate degree of edema (estimated dose of 15 mg detergent/kg body weight) [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Anesthetized dogs were exposed *via* a ventilator to particle sizes of 0.5 to 15 μm with an MMAD of 3 μm. Light microscopic examination of the lungs 4 hours after exposure to DOSS aerosol observed no grossly destructive effects on alveolar cells or lung architecture in exposed dogs. However, a decrease in pulmonary compliance was observed that the authors hypothesized was due to an increase in surface tension in the alveoli in the presence of detergent.

Pulmonary clearance studies using radiolabeled aerosol tracers have evaluated whether detergent effects on the surfactant layer lead to increased alveolar permeability. Inhalation exposure to DOSS enhanced the pulmonary clearance of radiolabeled diethylenetriamine pentaacetic acid (DTPA), a relatively small hydrophilic molecule, indicating an increased alveolar permeability after detergent exposure [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In most studies,

this effect on alveolar permeability was seen in the absence of effects on blood gas levels or pulmonary compliance that occurs with higher exposure, indicating that the increase in alveolar permeability is a sensitive effect of detergent aerosol. The effect was demonstrated to be concentration-related in rabbits exposed to multiple dilutions (0.125, 0.25, 0.5, and 2%) with a MMAD of 1.7 μm of the liquid detergent [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Studies also evaluated the clearance of a radiolabeled aerosol of albumin, a much larger molecule, which was enhanced by DOSS as well, but to a lesser degree than DTPA [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Wang *et al.* (1993) [ADDIN EN.CITE ADDIN EN.CITE.DATA] observed an increase in protein flux from plasma to alveolar space after DOSS inhalation in sheep, which was attributed to disruption of the alveolar lining and increased microvascular permeability. The increased alveolar permeability observed in these studies was hypothesized to be a result of increased alveolar surface tension, which may result in increased permeability by opening previously closed pores (through which solutes pass) in the membrane or by stretching already open pores [ADDIN EN.CITE ADDIN EN.CITE.DATA]. However, as previously mentioned, surfactants can disrupt cell membranes; thus, this mechanism may be an alternate explanation [ADDIN EN.CITE <EndNote><Cite><Author>Burden</Author><Year>2012</Year><RecNum>14727</RecNum><DisplayText>[1]</DisplayText><record><rec-number>14727</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596017177">14727</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Burden, D.W.</author></authors></contributors><titles><title>Guide to the Disruption of Biological Samples - 2012, Version 1.1.</title><secondary-title>Random Primers</secondary-

title></titles><periodical><full-title>Random Primers</full-title></periodical><pages>1-25</pages><number>12</number><dates><year>2012</year></dates><urls></urls></record></Cite></EndNote>].

Cationic Surfactants

In vivo studies

Three acute inhalation toxicity studies were identified for cationic surfactants; one study each for DDAC, dioctadecyldimethylammonium chloride (DODMAC), and BAC (CASRN 8001-54-5). DDAC, which is corrosive to the skin and severely damaging to the eye [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14786</RecNum><DisplayText>[69]</DisplayText><record><rec-number>14786</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596038295">14786</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Didecyldimethylammonium chloride, CASRN: 7173-51-5, EC number: 230-525-2, Skin irritation/corrosion</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/5864/7/4/2</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], was tested in rats (5/sex/dose, unspecified strain) exposed *via* inhalation to 0.05, 0.09, 0.13, 0.25, 1.36, or 4.54 mg/L (50, 90, 130, 250, 1,360, or 4,540 mg/m^3) for 2 hours with an observation period of 14 days (no additional exposure conditions reported). An LC_{50} of 0.07

mg/L was identified based on unspecified abnormalities identified in several organs including the lungs [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2006</Year><RecNum>14845</RecNum><DisplayText>[70]</DisplayText><record><rec-number>14845</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1597755265">14845</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Reregistration Eligibility Decision for Aliphatic Alkyl Quanternaries (DDAC)</title><secondary-title>Office of Chemical Safety and Pollution Prevention, Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>127, https://archive.epa.gov/pesticides/reregistration/web/pdf/ddac_red.pdf</pages><volume>EPA739-R-06-008</volume><dates><year>2006</year></dates><urls></urls></record></Cite></EndNote>]. A similar quaternary amine, DODMAC, which is irritating to the skin and causes serious damage to the eyes, was tested in albino rats (10 males, strain not specified) to the test substance (1:29 distilled water) *via* inhalation at 180 mg/L (180,000 mg/m^3) for one hour and observed for 14 days (no additional exposure conditions reported) [ADDIN EN.CITE <EndNote><Cite><Author>EURAR</Author><Year>2009</Year><RecNum>14787</RecNum><DisplayText>[71]</DisplayText><record><rec-number>14787</rec-number><foreign-

Commented [ST22]: Reference updated, per RT's flag that it was to the 4 week study, not the RED with LC50

keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596038841">14787</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EURAR</author></authors></contributors><titles><title>European Union Risk Assessment Report (EURAR), CAS No: 107-64-2, EINECS No: 203-508-2, dimethyldioctadecylammonium chloride (DODMAC)</title><secondary-title>European Commission, Joint Research Centre, Institute for Health and Consumer Protection (IHCP), former Toxicology and Chemical Substances (TCS) European Chemicals Bureau (ECB)</secondary-title></titles><periodical><full-title>European Commission, Joint Research Centre, Institute for Health and Consumer Protection (IHCP), former Toxicology and Chemical Substances (TCS) European Chemicals Bureau (ECB)</full-title></periodical><pages>123, https://echa.europa.eu/documents/10162/46f2f114-12ff-4af4-8da7-72148b6a202e</pages><volume>14</volume><dates><year>2009</year></dates><urls></urls></record></Cite></EndNote>]. No mortalities were reported and observed treatment-related clinical signs included preening, excessive masticatory (chewing) movements, excessive salivation stains, lacrimation, serosanguineous stains around the nose, and labored respiration. All animals appeared normal one day after dosing. The LD_{50}-LC_{50} (1 h) was > 180 mg/L. BAC, which is corrosive to the skin and causes severe eye damage [ADDIN EN.CITE ADDIN EN.CITE.DATA], was tested in female Wistar rats (5/group) exposed *via* nose-only inhalation to 37.6 and 53 mg/m^3 for 4 hours and observed for 14 days or exposed to 30.6 mg/m^3 for 6 hours and BALF was measured 18 hours post-exposure (MMAD and GSD not reported) [ADDIN EN.CITE <EndNote><Cite><Author>Swiercz</Author><Year>2008</Year><RecNum>14789</RecNum

><DisplayText>[73]</DisplayText><record><rec-number>14789</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596039305">14789</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Swiercz, R.</author><author>Halatek, T.</author><author>Wasowicz, W.</author><author>Kur, B.</author><author>Grzelińska, Z.</author><author>Majcherek, W.</author></authors></contributors><auth-address>Department of Toxicology and Carcinogenesis, Nofer Institute of Occupational Medicine, Łódź, Poland. radek@imp.lodz.pl</auth-address><titles><title>Pulmonary irritation after inhalation exposure to benzalkonium chloride in rats</title><secondary-title>Int J Occup Med Environ Health</secondary-title><alt-title>International journal of occupational medicine and environmental health</alt-title></titles><periodical><full-title>International journal of occupational medicine and environmental health</full-title><abbr-1>Int J Occup Med Environ Health</abbr-1></periodical><alt-periodical><full-title>International journal of occupational medicine and environmental health</full-title><abbr-1>Int J Occup Med Environ Health</abbr-1></alt-periodical><pages>157-63</pages><volume>21</volume><number>2</number><edition>2008/08/22</edition><keywords><keyword>Animals</keyword><keyword>Benzalkonium Compounds/administration & dosage/*toxicity</keyword><keyword>Female</keyword><keyword>Inhalation Exposure</keyword><keyword>Lung Diseases/*chemically induced/pathology</keyword><keyword>Organ Size/drug effects</keyword><keyword>Rats</keyword><keyword>Rats, Wistar</keyword></keywords><dates><year>2008</year></dates><isbn>1232-1087 (Print)
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num><urls></urls><electronic-resource-num>10.2478/v10001-008-0020-1</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]. The LC_{50} was reported to be approximately 53 mg/m^3 and BALF analysis reported increased inflammatory markers such as tumor necrosis factor (TNF)-a, interleukin (IL)-6. Indicators of lung damage, including increased LDH, total protein, and lung weight were also observed.

Three repeated dose inhalation studies of three different exposure durations were identified for DDAC: 14-day, 28-day, and 90-day.

In the 14-day study, male Sprague-Dawley rats were exposed *via* whole-body inhalation exposures to DDAC aerosols of 0.15 mg/m^3, 0.6 mg/m^3, and 3.6 mg/m^3 for 6 hours/day, 7 days/week [ADDIN EN.CITE <EndNote><Cite><Author>Lim</Author><Year>2014</Year><RecNum>14790</RecNum><DisplayText>[74]</DisplayText><record><rec-number>14790</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596039544">14790</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Lim, C. H.</author><author>Chung, Y. H.</author></authors></contributors><auth-address>Toxicity Research Team, Occupational Safety and Health Research Institute, KOSHA, Daejeon, Korea.</auth-address><titles><title>Effects of didecyldimethylammonium chloride on sprague-dawley rats after two weeks of inhalation exposure</title><secondary-title>Toxicol Res</secondary-title><alt-title>Toxicological research</alt-title></titles><periodical><full-title>Toxicol

Res</full-title><abbr-1>Toxicological research</abbr-1></periodical><alt-periodical><full-title>Toxicol Res</full-title><abbr-1>Toxicological research</abbr-1></alt-periodical><pages>205-10</pages><volume>30</volume><number>3</number><edition>2014/10/25</edition><keywords><keyword>Biocide</keyword><keyword>Didecyldimethylammonium chloride</keyword><keyword>Inhalation</keyword></keywords><dates><year>2014</year><pub-dates><date>Sep</date></pub-dates></dates><isbn>1976-8257 (Print)
1976-8257</isbn><accession-num>25343015</accession-num><urls></urls><custom2>PMC4206748</custom2><electronic-resource-num>10.5487/tr.2014.30.3.205</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]. The study authors reported an MMAD of 1.86 μm and a GSD of 2.75; however, individual values for each exposure concentration were not provided. Mild effects were noted in cell differential counts and cell damage parameters in BALF, in addition to inflammatory cell infiltration, and interstitial pneumonia at the medium and high exposures. The NOAEC was determined to be 0.15 mg/m^3.

In the intermediate exposure (4-week) study, male and female Sprague-Dawley rats (5 rats/sex/group) were exposed *via* dynamic nose-only inhalation to concentrations of 0, 0.08, 0.5, and 1.5 mg/m^3 DDAC (MMAD 1.4, 1.5, and1.9 μm, GSD 1.83, 1.86, and 1.87, density not reported) for 6 hours/day, 5 days/week [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2016</Year><RecNum>14732</RecNum><DisplayText>[10]</DisplayText><record><rec-number>14732</rec-number><foreign-

keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596018482">14732</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Subchronic Inhalation Toxicity Study of DDAC - Revised</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>25</pages><volume>HQ-OPP-2006-0338-0045</volume><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>] . Body weights were significantly reduced in the high exposure group (males only) on days 14, 21, and 25. Lung weights were increased in females in the mid- and high-concentration groups and in males in the high concentration group. BALF analysis indicated that, at the high concentration, neutrophils and eosinophils increased with a concomitant decrease in macrophages. Histopathological findings in the nasal cavity were reported as minimal to mild with increased mucus of the respiratory epithelium in males and females at all exposures and ulceration of the nasal cavity observed in males and females in the high concentration group only. In males, there was an increase in cell count and total protein across all exposures. In females, there was an increase in LDH across all concentrations, but the small sample size precluded establishing statistical significance for the effects. A conservative LOAEC of 0.08 mg/m^3 was previously identified by the Agency based on increased mucus of the respiratory epithelium and increased LDH; however, due to the mild effects and low number of animals/group, the effects were not statistically significant.[ADDIN EN.CITE

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<EndNote><Cite><Author>EPA</Author><Year>2016</Year><RecNum>14732</RecNum><DisplayText>[10]</DisplayText><record><rec-number>14732</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596018482">14732</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Subchronic Inhalation Toxicity Study of DDAC - Revised</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>25</pages><volume>HQ-OPP-2006-0338-0045</volume><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>]
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In the 13-week sub-chronic study, male and female Sprague-Dawley rats (10/group/sex) were exposed in whole body exposure chambers for 6 hours/day, 5 days/week [ADDIN EN.CITE <EndNote><Cite><Author>Kim</Author><Year>2017</Year><RecNum>14736</RecNum><DisplayText>[75]</DisplayText><record><rec-number>14736</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596018905">14736</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kim, Y. S.</author><author>Lee, S. B.</author><author>Lim, C. H.</author></authors></contributors><auth-address>Chronic Inhalation Toxicity Research Center, Chemicals Toxicity Research Bureau, Occupational Safety

and Health Research Institute, KOSHA, Daejeon, Korea.</auth-address><titles><title>Effects of Didecyldimethylammonium Chloride (DDAC) on Sprague-Dawley Rats after 13 Weeks of Inhalation Exposure</title><secondary-title>Toxicol Res</secondary-title><alt-title>Toxicological research</alt-title></titles><periodical><full-title>Toxicol Res</full-title><abbr-1>Toxicological research</abbr-1></periodical><alt-periodical><full-title>Toxicol Res</full-title><abbr-1>Toxicological research</abbr-1></alt-periodical><pages>7-14</pages><volume>33</volume><number>1</number><edition>2017/01/31</edition><keywords><keyword>Biocide</keyword><keyword>Didecyldimethylammonium chloride</keyword><keyword>Inhalation</keyword><keyword>Sub-chronic</keyword></keywords><dates><year>2017</year><pub-dates><date>Jan</date></pub-dates></dates><isbn>1976-8257 (Print)
1976-8257</isbn><accession-num>28133508</accession-num><urls></urls><custom2>PMC5266374</custom2><electronic-resource-num>10.5487/tr.2017.33.1.007</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]. The MMAD of the DDAC aerosol was 0.63 μm, 0.81 μm, and 1.65 μm, and the geometric standard deviations were 1.62, 1.65, and 1.65 in the low (0.11 ± 0.06 mg/m^3), the middle (0.36 ± 0.20 mg/m^3) and the high (1.41 ± 0.71 mg/m^3) exposure groups, respectively. Body weight influenced by exposure to DDAC with the mean body weight approximately 35% lower in the high exposure (1.41 ± 0.71 mg/m^3) male group and 15% lower in the high exposure (1.41 ± 0.71 mg/m^3) female group compared to that of the control group. Albumin and LDH were unaffected in the BALF. Lung weight was increased in females in the mid- and high-concentration groups and in males in the high

concentration group only, while inflammatory cell infiltration and interstitial pneumonia was observed in both the mid- and high-concentration groups. Tidal volume and minute volume were not significantly affected at any concentration. Severe histopathological symptoms such as proteinosis and/or fibrosis, were not reported. A NOAEC of 0.11 mg/m^3 was identified based on the increased lung weights in females and increase in inflammatory cells.

BAC was evaluated in a 2-week whole-body inhalation study in male and female Fischer rats (5/group/sex) to concentrations of 0.8, 4 and 20 mg/m^3 for 6 hours/day, 7 days/week [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Mean concentration of BAC in the whole-body exposure chambers of the T1 (0.8 mg/m^3), T2 (4 mg/m^3) and T3 (20 mg/m^3) groups during the exposure period was 0.84 ± 0.09, 4.01 ± 0.12, and 19.57 ± 0.97 mg/m^3, respectively; the MMAD of the aerosols was 1.614, 1.090, and 1.215 µm, respectively, and the GSD was 2.00, 1.86, and 1.51, respectively. The MMAD and GSD were confirmed to be within the range recommended by the OECD (OECD, 2018) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>14819</RecNum><DisplayText>[77]</DisplayText><record><rec-number>14819</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046851">14819</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Guidance Document on Inhalation Toxicity Studies, Series on Testing and Assessment, No. 39 (Second Edition)</title><secondary-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology,

Organization for Economic Cooperation and Development</secondary-title></titles><periodical><full-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</full-title></periodical><pages>106, https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2009)28/rev1&doclanguage=en</pages><volume>ENV/JM/MONO(2009)28/REV1</volume><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>]. Among the general signs observed during the exposure period, soiled perineal region, rales, and discharge were continuously observed during the 2-week recovery period.

Exposure-related effects were observed in the upper airway. Nasal discharge, rale, and deep respiration were observed in the high concentration, and nasal discharge was observed in the low and mid concentrations. In the nasal cavity, ulceration with suppurative inflammation, squamous metaplasia, and erosion with necrosis were observed in the respiratory epithelium and transitional epithelium of the male and female high concentrations.

In the lower airways, degeneration and regeneration of terminal bronchiolar epithelium, smooth muscle hypertrophy of bronchioloalveolar junction, and cell debris in the alveolar lumens were observed in the mid and high concentration male groups and high concentration dose female group. Hypertrophy and hyperplasia of mucous cells in the bronchi or bronchiole were observed in both males and females. The squamous metaplasia of the respiratory epithelium and transitional epithelium, mucinous cell hypertrophy and proliferation of the respiratory epithelium, mucinous cell metaplasia of the transitional epithelium in the nasal cavities, and

mucinous cell hypertrophy and proliferation of terminal bronchiole were considered adaptive changes after tissue injury. In the BALF analysis, the concentration of reactive oxygen species (ROS)/reactive nitrogen species (RNS), IL-1β, IL-6, and macrophage inflammatory protein (MIP)-2 decreased concentration-dependently at the end of the exposure period, which indicated oxidative damage, but did not show a concentration-dependent change at 4 weeks of recovery. The concentrations of TNF-α, IL-4, and transforming growth factor (TGF)-β did not show changes associated with test substance exposure. Relative lung weights were statistically significantly increased in males at the mid and high doses and in females at the high doses only. The study authors identified a LOAEC of 0.8 mg/m^3 based on effects in the nasal cavity.

Mechanistic studies

In vitro assays have demonstrated that cytotoxic effects of cationic surfactants have significantly greater toxicity to non-polarized than polarized mammalian cells [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In this study, cell viability as measured by LDH and MTT assays in non-polarized HeLa and fetal skin dendritic cells (FSDC) was more sensitive to the effects of different cationic surfactants of varying alkyl chain length and polar head groups than polarized cell lines Madin-Darby Canine Kidney (MDCK) and Caco-2. The cationic surfactant toxicity was shown to occur well below their CMC, and greater toxicity was observed with alkyl lengths of 10-12 than 14-16; however, this association was not strictly linear. In addition, the cationic surfactants with a larger polar head group (*i.e.*, benzalkonium) were 2-5 times more toxic than cationic surfactants with a more localized charge (*i.e.*, trimethylammonium).

The effects of BAC on cell viability, inflammatory response, and oxidative stress of human alveolar epithelial cells has been replicated *in vitro* using a dynamic culture condition that reflects the natural microenvironment of the lung to simulate the contraction and expansion of normal lungs [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Normal breathing levels were simulated (tidal volume 10%, 0.2Hz) through surface elongation of an elastic membrane in a dynamic culture system. This type of dynamic system provided easy control of exposure rate during the cell culture. The system assessed toxicity by culturing submerged cells with different BAC concentrations (0, 2, 5, 10, 20, and 40 μg/mL) under static and dynamic culture conditions. Following a 24-hr exposure to BAC, cellular metabolic activity, IL-8, and ROS levels were significantly affected, compared to untreated cells, when using either static or dynamic cell growth conditions. The dynamic culture system, which more closely mimics lung conditions, showed a higher toxic response to BAC as indicated by increased ROS levels.

Dose-Response Analysis: Quantitative Points of Departure (PODs)

The animal inhalation toxicity data identified by the literature search and PODs from the studies are summarized in [REF _Ref46931035 \h * MERGEFORMAT]. It should be emphasized that new information (*e.g.*, study data, POD derivation approaches, mechanistic information, *etc.*) may lead to updates/additions to this table. All of the identified data are from animal studies and therefore need to be extrapolated to estimate the human inhalation exposure [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><DisplayText>[18]</DisplayText><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal

Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. The exposure duration adjustment and DAF approaches were described above. The summary of RDDR inputs (*e.g.*, MMAD and GSD) and results are provided in [REF _Ref46931035 \h * MERGEFORMAT] for each of the toxicity studies from which PODs could be identified. However, other approaches may be considered relevant (*e.g.*, multiple-path particle dosimetry modeling [MPPD]).

For the nonionic surfactant, octylphenoxypolyethoxyethanol, the effects observed (increased lung weights, alveolar/bronchiolar epithelial hyperplasia and lung inflammation) are consistent with lung effects in the thoracic region; therefore, the RDDR of 0.812 was used to calculate the HEC. For the anionic surfactant, oleoylsarcosine, the effects were seen in multiple regions of the respiratory tract, including squamous metaplasia and epithelium proliferation and submucous acute inflammation at the base of the epiglottis and early stages of fibrosis in the alveoli walls. Therefore, the total respiratory tract RDDR (0.970) was used to calculate the HEC. In the 28-day

inhalation study with DDAC, effects were observed throughout the respiratory tract; therefore, the total respiratory tract RDDR (1.607) was used for calculating the HEC. Similarly, for the cationic surfactant, BAC histopathological cellular changes were observed in the nasal cavity and lungs, indicating the total respiratory tract RDDR (0.991) should be used to calculate the HEC. The RDDRs applied and HECs derived from the animal study PODs are provided in [REF _Ref46931035 \h * MERGEFORMAT].

Table [SEQ Table * ARABIC]. Inhalation Toxicity Points of Departure and Human Equivalent Concentrations (HEC) for Surfactants.

Surfactant Type	Chemical Substance	Inhalation Exposure Duration/Type	Study POD	Value (mg/m^3)	Reference	Density (g/cm^3) at 20 °C[1]	RDDR Model Input Parameters		RDDR[2]	HEC (mg/m^3)
							MMAD (μm)	GSD		
Nonionic	octylphenoxy polyethoxyethanol (CASRN 9002-93-1)	14-day, 6 hr/d, 5 d/wk; whole body	LOAEC	5.3	[ADDIN EN.CITE <EndNote><Cite><Author>MDEQ</Author><Year>2003</Year><RecNum>14731</RecNum><DisplayText>[8]</DisplayText><record><rec-number>14731</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0z	0.998 water vehicle	1.80	1.80	$RDDR_{ET}$ = 0.196 $RDDR_{TB}$ = 1.367 $RDDR_{PU}$ = 0.564 **$RDDR_{TH}$ = 0.812** $RDDR_{TOT}$ = 1.547	1.0 7.2 3.0 **4.4** 8.2

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					re0azr5ev earxfds0e rr5sr" timestamp ="159601 8112">14 731</key ></foreig n-keys><ref -type name="Jo urnal Article"> 17</ref- type><co ntributors ><authors ><author >MDEQ< /author>< /authors> </contribu tors><title s><title> To: Memo to File for Triton X- 100 (CAS # 9002- 93-1); From:					

					Gary Butterfield; Date: November 21,2003; Subject: Screening level for Triton X-100 (CAS # 9002-93-1)</title><secondary-title>Michigan Department of Environmental Quality (MDEQ)</secondary-title></titles><periodical><full-title>Michigan Department of					

					Environmental Quality (MDEQ)</full-title></periodical><pages>2</pages><dates><year>2003</year></dates><urls></urls></record></Cite></EndNote>]					
Anionic	oleoyl sarcosine (CASRN 110-25-8)	28-day, 6 hr/d, 5 d/wk; nose-only (OECD 412)	LOAEC	< 6	[ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14784</RecNum><DisplayText>[60]</DisplayText><recor	0.7893 ethanol vehicle	1.16	2.12	$RDDR_{ET}$ = 0.111 $RDDR_{TB}$ = 2.008 $RDDR_{PU}$ = 0.447 $RDDR_{TH}$ = 0.742 **$RDDR_{TOT}$ = 0.970**	< 0.6 < 12.0 < 2.7 < 4.5 **< 5.8**

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					d><rec-number>14784</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596036869">14784</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration					

					Dossier</author></authors></contributors><titles><title>N-methyl-N-[C18-(unsaturated)alkanoyl]glycine, CASRN: NA, EC number: 701-177-3, Repeated dose toxicity: Inhalation</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-					

					title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/21429/7/6/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]					
Cationic	DDAC	4-week, 6 hr/d, 5 d/wk; nose-only	LOAEC[3] (lung effects)	0.08	[ADDIN EN.CITE <EndNote><Cite><Author>E	NR	1.60	1.85	$RDDR_{ET}$ = 0.211 $RDDR_{TB}$ = 1.674 $RDDR_{PU}$ = 0.539 $RDDR_{TH}$ = 0.854 **$RDDR_{TOT}$ = 1.607**	0.02 0.13 0.04 0.07 **0.13**

[PAGE]

					PA</Author><Year>2016</Year><RecNum>14732</RecNum><DisplayText>[10]</DisplayText><record><rec-number>14732</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596018482">14732</key></foreign-keys><ref					

					-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Subchronic Inhalation Toxicity Study of DDAC - Revised</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental					

					Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>25</pages><volume>HQ-OPP-2006-					

					0338-0045</volume><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>]					
	BAC	14-day, 6 hr/d, 7 d/wk; whole body	LOAEC (nasal effects)	0.8	[ADDIN EN.CITE ADDIN EN.CITE.DATA]	0.998 water vehicle 2% dose solution	1.31	1.79	$RDDR_{ET} = 0.106$ $RDDR_{TB} = 1.988$ $RDDR_{PU} = 0.528$ $RDDR_{TH} = 0.815$ **$RDDR_{TOT} = 0.991$**	0.08 1.59 0.42 0.65 **0.79**

MMAD: Mass Median Aerodynamic Diameter of inhalation study aerosol, average values listed; GSD: Geometric Standard Deviation of the inhalation study aerosol, average values listed; RDDR: Regional Deposited Dose Ration; ET: Extrathoracic; TB: Tracheobronchial; PU: Pulmonary; TH: Thoracic = TB + PU; TOT = ET + TB + PU.

[1]Exact density of administered compounds not reported (NR); vehicle density was listed when provided.

[2]RDDR values are for male and female animals, whichever was lower, as calculated using RDDR.exe and described in the Supporting Information file at "Section 2 RD

[3]conservative estimate: effects were not statistically significant.

NA: Data not available or RDDR values could not be calculated from the available information.

Commented [ST24]: SM comment: "Question: is this RDDR animal to worker (heavier breathing rate) or to general population?"

Commented [ST25R24]: I double checked this, the RDDR is based on ratios for minute volume, regional fractional deposition, and a normalizing factor (surface area for POE effects)

Benchmark Margin of Exposure Analysis

The substances shown in [REF _Ref46931035 \h * MERGEFORMAT] provide representative examples of PODs that may be applied to new chemistries that meet the Surfactant Criteria, after evaluating whether the chemical substances in [REF _Ref46931035 \h * MERGEFORMAT] are appropriate toxicological analogues for read-across to the new chemical substance. Alternatively, the notifier may propose a different representative POD and/or analogue, if supported by scientific evidence. If a determination cannot be made on whether one of these chemical substances ([REF _Ref46931035 \h * MERGEFORMAT] or other representative analogue) is an appropriate toxicological analogue, then the relevant substance from [REF _Ref46931035 \h * MERGEFORMAT] should be identified as a comparator substance[3] for use in the Tiered-Testing Strategy, discussed below. Though the initial starting point for deriving a benchmark MOE is based on a composite of the default values of 10 for each of the individual values for UF_H, UF_A, and UF_L, refinements may be warranted based on dosimetric adjustments to the applied concentrations used for establishing the experimental PODs. As shown in [REF _Ref46931035 \h * MERGEFORMAT], the data-derived uncertainty factors were based on RDDRs that were used as DAFs to account for animal-to-human toxicokinetic differences.

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[3] A comparator substance is one that may possess similar properties to the new chemical substance and for which inhalation toxicity data are available. EPA may "read-across" the toxicity data from the comparator substance to the new chemical substance when no other information is available. The tiered-testing approach for this category is designed to determine whether this practice may be refined or supported by additional data. As such, the comparator substance should be used in side-by-side testing in Tiers I-III with a new chemical substance to aid with interpreting the test results of the new chemical substance.

In the case of surface-active substances meeting the Surfactant Criteria, EPA has recently adopted a generalized approach that has historically been applied on a case-by-case basis for chemical substances, in recognition that surface-active effects that lead to irritation/corrosion do not require absorption, metabolism, distribution, or elimination (ADME) (See, *e.g.*, EPA, 2020 [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2020</Year><RecNum>14794</RecNum><DisplayText>[80]</DisplayText><record><rec-number>14794</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596040494">14794</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Hazard Characterization of Isothiazolinones in Support of FIFRA Registration Review</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>84, https://www.regulations.gov/contentStreamer?documentId=EPA-HQ-OPP-2013-0605-0051&contentType=pdf</pages><volume>EPA-HQ-OPP-2013-0605-0051</volume><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]). In the context of this publication, irritation/corrosion include those effects in the respiratory tract that lead to inflammation, hyperplasia, and metaplasia. For chemical substances that act *via* a surface-active adverse outcome pathway (AOP) [ADDIN EN.CITE <EndNote><Cite><Author>Sorli</Author><Year>2020</Year><RecNum>14800</RecNum><

DisplayText>[81]</DisplayText><record><rec-number>14800</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041625">14800</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sorli, J. B.</author></authors></contributors><titles><title>Lung Surfactant Function Disruption Leading to Acute Inhalation Toxicity</title><secondary-title>AOPWiki</secondary-title></titles><periodical><full-title>AOPWiki</full-title></periodical><pages>https://aopwiki.org/aops/302</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], the default values for UF_H and UF_A are reduced to 3 (*i.e.*, $10^{0.5}$ or 3.162) to account for the uncertainty/variability for toxicodynamics, whereas the toxicokinetic component is reduced to 1. In order to apply these reductions, the following criteria must be established:

1. A description of the AOP,
2. A discussion of why the AOP is unlikely or likely to differ between humans, in the case of UF_H, or between animals in comparison to humans, in the case of UF_A, and
3. A discussion as to why the ADME of the chemical substance is addressed by the use of dosimetry modeling.

When the above criteria are met, application of the appropriate DAF (*e.g.*, the RDDR for particles) should still be applied, given that deposition is the most appropriate dose metric for assessing acute/subacute effects from surface-active agents. However, since the DAF accounts

for the toxicokinetic component of UF_A, the remaining value of 3 (*i.e.*, $10^{0.5}$ or 3.16) should be retained for the toxicodynamics component of the UF_A.

Based on these information and criteria, the following composite values are appropriate to describe intra- and interspecies variability (*i.e.*, $UF_H \times UF_A$):

UF_H = 10 or 3: The default value of 10 should be applied when the available information does not support each of the above criteria. If the available information supports all three of the above criteria, then a value of 3 may be applied. The reduced value represents a reduction in the toxicokinetic component of this UF to 1, with the remaining value of 3 accounting for the toxicodynamic component.

UF_A = 10 or 3: The default value of 10 should be applied when the available information does not support the application of dosimetric adjustments for quantifying an HEC or when the available information does not support each of the above three criteria. If the available information allows derivation of an HEC and/or application of the above criteria, then a value of 3 may be applied, which represents a reduction in the toxicokinetic component to 1 and application of a value of 3 for toxicodynamics.

UF_L = 10 or 1: If the POD from the experimental study is based on a LOAEC, then a default value of 10 should be applied, unless there is information to support that a reduced value is warranted. If the experimental data are amenable to benchmark dose modeling, a BMCL should be calculated and a value of 1 should be applied for this area of uncertainty.

The above considerations and approaches support the application of a benchmark MOE ranging from 10 (*i.e.*, $10^{0.5} \times 10^{0.5} \approx 10$) to 1,000 depending on the chemical substance identified as an appropriate toxicological analogue and available data on the new chemical substance. In those instances where the data are too limited to determine when one of the chemical substances is appropriate for extrapolating the hazards to the new chemical substance, experimental testing should be performed to aid with informing the quantitative assessment, as discussed under the Tiered-Testing Strategy.

Uncertainties and Limitations

The assessment framework outlined includes a number of uncertainties and limitations, including those associated with extrapolating the hazards identified from the chemical substances shown in [REF _Ref46931035 \h * MERGEFORMAT]. Uncertainties associated with using animal studies to estimate human toxicity are recognized and methods are presented to reduce extrapolation uncertainties [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2014</Year><RecNum>14795</RecNum><DisplayText>[82]</DisplayText><record><rec-number>14795</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596040729">14795</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Guidance on Grouping of Chemicals, Second Edition, Series on Testing & Assessment</title><secondary-title>Environment Directorate, Joint Meeting of the Chemicals

Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</secondary-title></titles><periodical><full-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</full-title></periodical><pages>141, http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2014)4&doclanguage=en</pages><volume>ENV/JN/MONO(2014)4</volume><dates><year>2014</year></dates><urls></urls></record></Cite></EndNote>]. Procedures for the adjustment of exposure durations for inhalation exposures and application of DAFs to derive HECs are well-established procedures for reducing uncertainties associated with the toxicokinetic aspects of animal-to-human extrapolation factors and derivation of benchmark MOEs (*i.e.*, type and magnitude of uncertainty factors) [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2002</Year><RecNum>14743</RecNum><DisplayText>[17, 18]</DisplayText><record><rec-number>14743</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019884">14743</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>A Review of the Reference Dose and Reference Concentration Processes</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>192, https://www.epa.gov/sites/production/files/2014-

12/documents/rfd-final.pdf</pages><volume>EPA/630/P-02/002F</volume><dates><year>2002</year></dates><urls></urls></record></Cite><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. Likewise, EPA has recommended that BMD modeling be employed whenever possible to identify a POD and to reduce uncertainties associated with using a LOAEL from a toxicity study.

Given the small number of chemical substances that meet the Surfactant Criteria that have concentration-response inhalation toxicity data, the applicability of the chemical substances in [REF _Ref46931035 \h * MERGEFORMAT] to new chemical substances needs to be carefully considered, with attention given to the influence of additional functional groups on the toxicity of the new chemical substance. Additionally, the risk assessors should consider if a different

comparator substance and/or POD may be more appropriate (*e.g.*, based on new scientific information of the new chemical substance profile). Risk assessors should consider the surface tension and CMC criteria ([REF _Ref47613375 \h * MERGEFORMAT]) compared to these measurements for the new chemical substance and the influence of the presence or absence of additional functional groups on these criteria (*e.g.*, would a particular functional group increase or decrease toxicity, for example by another mechanism of action). If such structural differences are judged not to significantly influence properties and toxicity, such that the new chemical substance is expected to have comparable or lower toxicity, the hazard(s) and risk(s) should be characterized using the chemical substance as a toxicological analogue to the new chemical substance. Of course, uncertainties regarding this extrapolation should be acknowledged in the risk characterization.

For instances where the notifier of the new chemical substance and/or EPA is unable to conclude that ~~one of the~~ chemical substances ~~in~~ ([REF _Ref46931035 \h * MERGEFORMAT] or other relevant analogue) is comparable to or represents an acceptable toxicological analogue to the new chemical substance, then the Tiered-Testing Strategy provided could be used to determine whether the new chemical substance has lower, comparable, or higher toxicity to the relevant chemical substance in [REF _Ref46931035 \h * MERGEFORMAT], as a comparator substance and not as a toxicological analogue. Prior to conducting such testing, the scientific basis for selecting the comparator substance to the new chemical substance should be understood and a rationale provided as to why the comparator substance will be used for testing.

Use of New Approach Methods (NAMs) and *In Vitro* Testing Strategies to Reduce or Replace Vertebrate Testing

The amended TSCA requires EPA to reduce reliance on animal testing using methods and strategies that "provide information of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment" [ADDIN EN.CITE <EndNote><Cite><Author>U.S.C.</Author><Year>2016</Year><RecNum>14796</RecNum><DisplayText>[83]</DisplayText><record><rec-number>14796</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041048">14796</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>U.S.C.</author></authors></contributors><titles><title>Title 15-Commerce and Trade, Chapter 53-Toxic Substances Control, Subchapter I-Control of Toxic Substances</title><secondary-title>United States Code (U.S.C.)</secondary-title></titles><periodical><full-title>United States Code (U.S.C.)</full-title></periodical><pages>https://uscode.house.gov/view.xhtml?path=/prelim@title15/chapter53&edition=prelim</pages><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>]. Moreover, the amended TSCA requires entities undertaking voluntary testing for submission to EPA to first "…attempt to develop the information by means of an alternative test method or strategy …before conducting new vertebrate testing…" [ADDIN EN.CITE <EndNote><Cite><Author>U.S.C.</Author><Year>2016</Year><RecNum>14796</RecNum><DisplayText>[83]</DisplayText><record><rec-number>14796</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041048">14796</key></foreign-keys><ref-type name="Journal

Article">17</ref-type><contributors><authors><author>U.S.C.</author></authors></contributors><titles><title>Title 15-Commerce and Trade, Chapter 53-Toxic Substances Control, Subchapter I-Control of Toxic Substances</title><secondary-title>United States Code (U.S.C.)</secondary-title></titles><periodical><full-title>United States Code (U.S.C.)</full-title></periodical><pages>https://uscode.house.gov/view.xhtml?path=/prelim@title15/chapter53&edition=prelim</pages><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>]. Additionally, in 2019, EPA was directed to prioritize efforts to use NAMs to reduce animal testing [ADDIN EN.CITE <EndNote><Cite><Author>Wheeler</Author><Year>2019</Year><RecNum>14797</RecNum><DisplayText>[84]</DisplayText><record><rec-number>14797</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041176">14797</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wheeler, A.R.</author></authors></contributors><titles><title>Directive to Prioritize Effects to Reduce Animal Testing</title><secondary-title>United States Environmental Protection Agency</secondary-title></titles><periodical><full-title>United States Environmental Protection Agency</full-title></periodical><pages>3, https://www.epa.gov/sites/production/files/2019-09/documents/image2019-09-09-231249.pdf</pages><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>]. Multiple NAMs exist which can be used to assess hazards and risks of new chemical substances that meet the Surfactant Criteria, including validated OECD methods for *in vitro* irritation testing and *in vitro* methods to specifically assess respiratory toxicity. Several methods

are described within a tiered-testing strategy recognizing that these assays are provided as examples and the development of NAMs is advancing rapidly. As such, the NAMs included here should not be considered all-inclusive or a final compilation. EPA strongly encourages the development and use of NAMs, particularly to reduce or replace the use of vertebrate animals and is open to considering and discussing additional NAMs with PMN submitters during a pre-notice consultation [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2020</Year><RecNum>14829</RecNum><DisplayText>[85]</DisplayText><record><rec-number>14829</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596098792">14829</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Schedule a Pre-Submission Meeting, Reviewing New Chemicals under the Toxic Substances Control Act (TSCA)</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>https://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/forms/program-contacts-and</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>].

In the interest of reducing or replacing vertebrate testing and designing a scientifically robust testing approach, when a surfactant is determined to be respirable, EPA encourages evaluating its

potential to cause pulmonary toxicity using an AOP approach. The OECD provides "An AOP is an analytical construct that describes a sequential chain of causally linked events at different levels of biological organization that lead to an adverse health or ecotoxicological effect" and that "AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning" [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2020</Year><RecNum>14798</RecNum><DisplayText>[86]</DisplayText><record><rec-number>14798</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041285">14798</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Adverse Outcome Pathways, Molecular Screening and Toxicogenomics</title><secondary-title>Organization for Economic Cooperation and Development (OECD)</secondary-title></titles><periodical><full-title>Organization for Economic Cooperation and Development (OECD)</full-title></periodical><pages>http://www.oecd.org/env/ehs/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>].

Representative key elements of AOPs are the molecular initiating events (MIEs), cellular level events (CLEs), organ or tissue level events (OLEs), and organism consequent events (OCEs). For surfactants, the initial key event is proposed to be the interaction of the substance with epithelial lining fluid or lung-surfactant (MIE) and/or the molecular interaction of the substance

itself with cell membranes of the epithelium in the respiratory tract (MIE), resulting in the disruption of lung cells due to loss of lung cell surfactant function (CLE) and/or the loss of membrane integrity (CLE). These initial events may lead to different OLEs (*e.g.*, cytotoxicity and perturbation of airway epithelial cells, alveolar collapse, loss of barrier function, blood extravasation, and impaired oxygenation of blood), which may finally lead to organism consequences (OCE) (*e.g.*, pneumonia, limited lung function by chronic obstruction (COPD), fibroses, *etc*.). AOPs in various stages of development are useful for different purposes and an AOP may still be useful even if it has not been formally evaluated by the OECD.

An AOP can be used to help design a testing strategy and to identify NAMs that can query the key events leading up to the adverse outcome. As an example, using the respiratory irritant chlorothalonil, Syngenta Crop Protection applied a NAM for the assessment of inhalation toxicology based on AOP [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The approach involved derivation of the POD from an *in vitro* assay and the integration of the *in vitro* POD for calculation of HECs for the inhalation risk assessment. Similar approaches can be used for surfactants where *in vitro* systems may be used to investigate specific key events in the AOP and confirm that a new chemical substance fits within the boundaries of the Surfactant Category or a sub-category and therefore may act like a surfactant (group assignment *via* similar AOP) and/or if other substance specific properties lead to a predominant type of key event within the AOP. Further, *in vitro* tests may deliver information while avoiding *in vivo* testing or provide helpful information on dose-selection for *in vivo* testing, if needed. Some *in vitro* tests, such as by capillary surfactometer, may be useful in preliminary screening of chemicals to be tested, but do not by themselves constitute adequate tests for acute pulmonary effects of these chemicals. This

information should be taken into consideration within the design of additional tests. These assays can be used as part of a weight of scientific evidence evaluation to determine whether animal testing is needed or if a POD can be determined for risk assessment purposes without the use of animals. These tests may also provide insight on one or more components of the AOP.

Based on the surfactant AOP framework under development [ADDIN EN.CITE <EndNote><Cite><Author>Sorli</Author><Year>2020</Year><RecNum>14800</RecNum><DisplayText>[81]</DisplayText><record><rec-number>14800</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041625">14800</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sorli, J. B.</author></authors></contributors><titles><title>Lung Surfactant Function Disruption Leading to Acute Inhalation Toxicity</title><secondary-title>AOPWiki</secondary-title></titles><periodical><full-title>AOPWiki</full-title></periodical><pages>https://aopwiki.org/aops/302</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], a number of different types of *in vitro* test methods, summarized in [REF _Ref46931271 \h * MERGEFORMAT], may provide potentially useful information for informing the various elements of the surfactant AOP. Clippinger *et al*. (2018) [ADDIN EN.CITE ADDIN EN.CITE.DATA] have also described a decision tree and potential MIEs and key events that can be used to design pathway-based approaches for *in vitro* testing of acute inhalation exposures.

Table [SEQ Table * ARABIC]. *In Vitro* Test Methods and New Approach Methods That May Be Useful for Evaluating Chemicals for Inclusion in Surfactant AOP and Category.

Surfactant AOP	*Information on AOP*	*In Vitro* Assay	Test System
Molecular Initiating Events (MIEs)	MIE for interaction with pulmonary surfactant/loss of function	*In Vitro* Respiratory Toxicity Assays	• *In vitro* lung surfactant interaction, *e.g.*, as described by Sorli *et al.* (2018) [ADDIN EN.CITE ADDIN EN.CITE.DATA]
	MIE for disruption of cell membrane components and interaction /penetration through cell membrane	*Hemoglobin Denaturation Assay, Liposome Assay, and In Vitro/Ex Vivo* Irritation Assays	• Hemoglobin denaturation assay, *e.g.*, as described by Hayashi *et al.* (1994) [ADDIN EN.CITE <EndNote><Cite><Author>Hayashi</Author><Year>1994</Year><RecNum>14838</RecNum><DisplayText>[91]</DisplayText><rec id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596732926">14838</key></foreign-keys><ref-type name="Journal Article"> H.</author><author>Fukuda, T.</author><author>Tamura, U.</author><author>Kato, S.</author></authors></contributors><auth-addre address><titles><title>Multivariate factorial analysis of data obtained in seven in vitro test systems for predicting eye irritancy</title><sec published in association with BIBRA</alt-title></titles><periodical><full-title>Toxicology in vitro : an international journal published in periodical><full-title>Toxicology in vitro : an international journal published in association with BIBRA</full-title><abbr-1>Toxicol In V 20</pages><volume>8</volume><number>2</number><edition>1994/04/01</edition><dates><year>1994</year><pub-dates><date>Ap num>20692908</accession-num><urls></urls><electronic-resource-num>10.1016/0887-2333(94)90185-6</electronic-resource-num><re provider><language>eng</language></record></Cite></EndNote>]. • Liposome assay, *e.g.*, as described by Kapoor *et al.* (2009) [ADDIN EN.CITE <EndNote><Cite><Author>Kapoor</Author><Year>2009 number>14834</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596539300" type><contributors><authors><author>Kapoor, Y.</author><author>Howell, B. A.</author><author>Chauhan, A.</author></authors>< Florida 32611, USA.</auth-address><titles><title>Liposome assay for evaluating ocular toxicity of surfactants</title><secondary-title>In science</alt-title></titles><periodical><full-title>Investigative ophthalmology & visual science</full-title><abbr-1>Invest Ophthalm science</full-title><abbr-1>Invest Ophthalmol Vis Sci</abbr-1></alt-periodical><pages>2727-35</pages><volume>50</volume><numb Diseases/chemically induced</keyword><keyword>Corneal Diseases/chemically induced</keyword><keyword>*Diagnostic Techniques Ophthalmological</keyword><keyword>Fluoresceins/*metabolism</keyword><keyword>Fluorescent Dyes/*metabolism</keyword><ke Measurements</keyword><keyword>Models, Theoretical</keyword><keyword>Permeability/drug effects</keyword><keyword>Surfac dates><date>Jun</date></pub-dates></dates><isbn>0146-0404</isbn><accession-num>19168898</accession-num><urls></urls><electr provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>].

			• OECD *In vitro/ex vivo* eye irritation tests for penetrance, *e.g.*, Reconstructed human Cornea-like Epithelium (RhCE) (OECD 492) [ADDI <EndNote><Cite><Author>OECD</Author><Year>2019</Year><RecNum>14803</RecNum><DisplayText>[93]</DisplayText><reco id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596043912">14803</key></foreign-keys><ref-type name="Journal Article"> type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Reconstructed human Cornea-like Epithe irritation or serious eye damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><period https://www.oecd-ilibrary.org/docserver/9789264242548- en.pdf?expires=1596044765&id=id&accname=guest&checksum=C972EFF7A2459C37BF048A1BDC82F2D4</pages><v Bovine Corneal Opacity and Permeability Test (OECD 437) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>202 number>14802</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596043719"> type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Bovine Corneal Opacity And Permeabili Requiring Classification For Eye Irritation Or Serious Eye Damage</title><secondary-title>OECD Guidelines for the Testing of Chemica Chemicals</full-title></periodical><pages>28, https://www.oecd-ilibrary.org/docserver/9789264203846- en.pdf?expires=1596044549&id=id&accname=guest&checksum=6B06BCD6D113D26A04C508907C001D91</pages><v Isolated Chicken Eye Test (OECD 438) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044057">14804</key></forei type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Isolated chicken eye test method for iden irritation or serious eye damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><period https://www.oecd-ilibrary.org/docserver/9789264203860- en.pdf?expires=1596044906&id=id&accname=guest&checksum=37A7598040CEC8996E712477F0A603D7</pages><vol *etc*.
Cellular Level Events (CLEs)	CLE for loss of membrane integrity/general cytotoxicity	*In Vitro/Ex Vivo* Cytotoxicity Assays	• OECD *In vitro/ex vivo* eye irritation tests for cytotoxicity, *e.g.*, Reconstructed human Cornea-like Epithelium (RhCE) (OECD 492) [ADD <EndNote><Cite><Author>OECD</Author><Year>2019</Year><RecNum>14803</RecNum><DisplayText>[93]</DisplayText><reco id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596043912">14803</key></foreign-keys><ref-type name="Journal Article"> type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Reconstructed human Cornea-like Epithe irritation or serious eye damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><period https://www.oecd-ilibrary.org/docserver/9789264242548- en.pdf?expires=1596044765&id=id&accname=guest&checksum=C972EFF7A2459C37BF048A1BDC82F2D4</pages><v Bovine Corneal Opacity and Permeability Test (OECD 437) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>202 number>14802</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596043719"> type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Bovine Corneal Opacity And Permeabili Requiring Classification For Eye Irritation Or Serious Eye Damage</title><secondary-title>OECD Guidelines for the Testing of Chemica Chemicals</full-title></periodical><pages>28, https://www.oecd-ilibrary.org/docserver/9789264203846- en.pdf?expires=1596044549&id=id&accname=guest&checksum=6B06BCD6D113D26A04C508907C001D91</pages><v

			Isolated Chicken Eye Test (OECD 438) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044057">14804</key></forei type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Isolated chicken eye test method for iden irritation or serious eye damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><period https://www.oecd-ilibrary.org/docserver/9789264203860-en.pdf?expires=1596044906&id=id&accname=guest&checksum=37A7598040CEC8996E712477F0A603D7</pages><vol *etc*.
			• Cell membrane integrity test (LDH-cytotoxicity assay), MTT assay, TEER, ATP, or lysosomal membrane integrity test. • BALB/c3T3/A549 lung cells neutral red uptake (NRU) cytotoxicity test, a test for basal cytotoxicity (ICCVAM, 2006) [ADDIN EN.CITE <EndNote><Cite><Author>ICCVAM</Author><Year>2006</Year><RecNum>14805</RecNum><DisplayText>[96]</DisplayText><r id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044231">14805</key></foreign-keys><ref-type name="Journal Article"> type><contributors><authors><author>ICCVAM</author></authors></contributors><titles><title>In vitro Cytotoxicity Test Methods fo title>ICCVAM Test Method Evaluation Report</secondary-title></titles><periodical><full-title>ICCVAM Test Method Evaluation Repo https://ntp.niehs.nih.gov/iccvam/docs/acutetox_docs/brd_tmer/at-tmer-complete.pdf</pages><volume>NIH Publication No. 07-4519</vol
Organ or Tissue Level Events (OLEs)	OLE for tissue level events	Human organotypic Airway Epithelial Cultures	• 3D constructs of human-derived cell cultures of differentiated airway epithelial cells (*e.g.*, EpiAirway™, MucilAir™, SmallAir™, EpiAlve
	OLE for tissue level events	Specific *Ex Vivo* Respiratory Toxicity Assays	• Precision-cut lung slice test, *e.g.,* as described by Hess *et al.* (2016) [ADDIN EN.CITE ADDIN EN.CITE.DATA] and Neuhaus *et a*

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The surfactant AOP is hypothesized to consist of two MIEs that may be informed by *in vitro* assays to determine whether a particular chemistry causes adverse effects on the epithelial lining fluid (ELF) or pulmonary surfactant system (MIE #1) or cytotoxicity to airway epithelial or pulmonary cell membranes (MIE #2), or both. For MIE #1, Sorli *et al.* (2017) [ADDIN EN.CITE ADDIN EN.CITE.DATA] developed an *in vitro* lung surfactant interaction assay that specifically measures whether the substance interferes with lung surfactant function. The assay was initially developed for predicting the effect of waterproofing agents that were shown to be acutely toxic to mice. The authors noted that it may be overly conservative for some substances. Nevertheless, this assay investigated a basic principle (*e.g.*, MIE #1) which may also be relevant for some types of surfactants. For MIE #2, the hemoglobin denaturation and liposome assays and *in vitro* eye irritation assays do not directly measure effects on membranes of pulmonary cells; however, these assays have been shown to provide indirect lines of evidence as a screening approach for determining the ability of surfactants to interact with cellular membrane components and cell membrane penetration. For example, Hayashi *et al.* (1995) [ADDIN EN.CITE <EndNote><Cite><Author>Hayashi</Author><Year>1995</Year><RecNum>14833</RecNum><DisplayText>[100]</DisplayText><record><rec-number>14833</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596539110">14833</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hayashi, T.</author><author>Itagaki, H.</author><author>Fukuda, T.</author><author>Tamura, U.</author><author>Sato,

Y.</author><author>Suzuki, Y.</author></authors></contributors><auth-address>Shiseido Research Center, Yokohama, Japan.</auth-address><titles><title>Hemoglobin denaturation caused by surfactants</title><secondary-title>Biol Pharm Bull</secondary-title><alt-title>Biological & pharmaceutical bulletin</alt-title></titles><alt-periodical><full-title>Biological & Pharmaceutical Bulletin</full-title><abbr-1>Biol. Pharm. Bull.</abbr-1></alt-periodical><pages>540-3</pages><volume>18</volume><number>4</number><edition>1995/04/01</edition><keywords><keyword>Chromatography, High Pressure Liquid</keyword><keyword>Circular Dichroism</keyword><keyword>Hemoglobins/*chemistry</keyword><keyword>Irritants/pharmacology</keyword><keyword>Protein Denaturation/drug effects</keyword><keyword>Sodium Dodecyl Sulfate/pharmacology</keyword><keyword>Spectrophotometry</keyword><keyword>Structure-Activity Relationship</keyword><keyword>Surface-Active Agents/*pharmacology</keyword><keyword>Taurine/analogs & derivatives/pharmacology</keyword></keywords><dates><year>1995</year><pub-dates><date>Apr</date></pub-dates></dates><isbn>0918-6158 (Print)
0918-6158</isbn><accession-num>7655423</accession-num><urls></urls><electronic-resource-num>10.1248/bpb.18.540</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>] showed that charged surfactant molecules can interfere with charged side chains of the hemoglobin protein. These interactions lead to disruption of the three-dimensional (3D) structure of hemoglobin, causing a change in light absorbance that can be measured. Increasing concentrations of SDS and sodium

lauroylmethyltaurate (LMT) were tested in this assay and showed concentration dependent increases in hemoglobin denaturation, which correlated with irritation effects in the Draize eye test [ADDIN EN.CITE ADDIN EN.CITE.DATA].

The liposome assay can be used to assess disruption of the lipid bilayer of the membrane from interaction with surfactant chemistries. Kapoor *et al.* (2009) [ADDIN EN.CITE <EndNote><Cite><Author>Kapoor</Author><Year>2009</Year><RecNum>14834</RecNum><DisplayText>[92]</DisplayText><record><rec-number>14834</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596539300">14834</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kapoor, Y.</author><author>Howell, B. A.</author><author>Chauhan, A.</author></authors></contributors><auth-address>Department of Chemical Engineering, University of Florida, Gainesville, Florida 32611, USA.</auth-address><titles><title>Liposome assay for evaluating ocular toxicity of surfactants</title><secondary-title>Invest Ophthalmol Vis Sci</secondary-title><alt-title>Investigative ophthalmology & visual science</alt-title></titles><periodical><full-title>Investigative ophthalmology & visual science</full-title><abbr-1>Invest Ophthalmol Vis Sci</abbr-1></periodical><alt-periodical><full-title>Investigative ophthalmology & visual science</full-title><abbr-1>Invest Ophthalmol Vis Sci</abbr-1></alt-periodical><pages>2727-35</pages><volume>50</volume><number>6</number><edition>2009/01/27</edition><keywords><keyword>Conjunctival Diseases/chemically induced</keyword><keyword>Corneal Diseases/chemically induced</keyword><keyword>*Diagnostic Techniques,

Ophthalmological</keyword><keyword>Fluoresceins/*metabolism</keyword><keyword>Fluor
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Dyes/*metabolism</keyword><keyword>Humans</keyword><keyword>*Liposomes</keywor
d><keyword>Luminescent Measurements</keyword><keyword>Models,
Theoretical</keyword><keyword>Permeability/drug effects</keyword><keyword>Surface-
Active Agents/*toxicity</keyword></keywords><dates><year>2009</year><pub-
dates><date>Jun</date></pub-dates></dates><isbn>0146-0404</isbn><accession-
num>19168898</accession-num><urls></urls><electronic-resource-num>10.1167/iovs.08-
2980</electronic-resource-num><remote-database-provider>NLM</remote-database-
provider><language>eng</language></record></Cite></EndNote>] measured the release of calcein dye from liposomes following exposure to various surfactants and showed a positive correlation with these findings and data from the Draize eye test. The hemoglobin denaturation and liposomal assays were both optimized and validated against eye irritation data; therefore, these assays may provide an opportunity to evaluate the effects of surfactants on the respiratory tract. Further *in vitro* testing of known surfactants with existing data alongside new chemical substances will help benchmark the results. Nonetheless, these assays are envisioned to be useful for understanding the potential for a new surfactant substance to act *via* MIE #2 in the respiratory tract.

The use of *ex vivo* eye irritation studies may provide indirect measures of surfactants on cell membranes, which may be relevant to the effects observed from comparator substances in the respiratory tract. For example, Bader *et al*. (2013) [ADDIN EN.CITE
<EndNote><Cite><Author>Bader</Author><Year>2014</Year><RecNum>14807</RecNum>

Commented [HT27]: I started new para here

<DisplayText>[102]</DisplayText><record><rec-number>14807</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044694">14807</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bader, J.E.</author><author>Norman, K.G.</author><author>Raabe, H.</author></authors></contributors><titles><title>Predicting Ocular Irritation of Surfactants Using the Bovine Corneal Opacity and Permeability Assay</title><secondary-title>Insitute for In Vitro Sciences, Inc., Gaithersburg, M.D.</secondary-title></titles><periodical><full-title>Insitute for In Vitro Sciences, Inc., Gaithersburg, M.D.</full-title></periodical><pages>https://iivs.org/wp-content/uploads/2018/08/iivs_poster_predicting-ocular-irritation-of-surfactants-using-the-bovine-corneal-opacity-and-permeability-assay.pdf</pages><dates><year>2014</year></dates><urls></urls></record></Cite></EndNote>] reported that the Bovine Corneal Opacity and Permeability (BCOP) assay was effective at demonstrating that nonionic (*i.e.*, octylphenoxypolyethoxyethanol), anionic (*i.e.*, SDS), and cationic (*i.e.*, BAC) substances cause irritation to the eye; however, the authors also noted that the endpoints evaluated in this assay should be carefully assessed independently. The permeability score was more predictive of eye irritation than the ocular opacity score for octylphenoxypolyethoxyethanol and SDS, whereas with BAC, the opacity score was more predictive of eye irritation than the permeability score. Therefore, a systematic investigation of opacity and permeability measures with surfactants using this approach may be helpful with elucidating MIE #2 of the AOP. In addition, information on the potential of a substance to cause skin irritation (*e.g.*, OECD TG 439 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2020</Year><RecNum>14808</RecNum>

<DisplayText>[103]</DisplayText><record><rec-number>14808</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044884">14808</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Reconstructed Human Epidermis Test Method, In vitro Skin Irritation</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>26, https://www.oecd-ilibrary.org/docserver/9789264242845-en.pdf?expires=1596045726&id=id&accname=guest&checksum=2580E92A5C889D0DD65599260E7866D3</pages><volume>439</volume><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]) and/or skin corrosion (*e.g.*, OECD TG 431 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2019</Year><RecNum>14809</RecNum><DisplayText>[104]</DisplayText><record><rec-number>14809</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044976">14809</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>In Vitro Skin Corrosion: Reconstructed Human Epidermis (RhE) Test Method</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>29, https://www.oecd-ilibrary.org/docserver/9789264264618-

en.pdf?expires=1596045820&id=id&accname=guest&checksum=E3EE55CBAA FAF0432EAD109F1B39ECF0</pages><volume>431</volume><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>]) *in vitro*, can provide supporting evidence of the potential for a substance to cause similar irritant or corrosive effects in respiratory tract cells. Corrosion effects mediated by pH extremes should be distinguished from necrosis effects *via* membrane disruption, demonstrated by DDAC that causes tissue effects in inhalation studies despite having a neutral pH value of 6.8-6.9 [ADDIN EN.CITE <EndNote><Cite><Author>Sigma-Aldrich</Author><Year>2020</Year><RecNum>14810</RecNum><DisplayText>[105]</DisplayText><record><rec-number>14810</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596045132">14810</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sigma-Aldrich</author></authors></contributors><titles><title>Safety Data Sheet, Product name: Didecyldimethylammonium chloride, Version 8.1, Revision Date: 03/28/2020, Print Date: 05/29/2020</title></titles><pages>9, https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=34466&brand=SIAL&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsial%2F34466%3Flang%3Den</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>].

CLEs

Several *in vitro/ex vivo* assays may determine whether a new chemical substance is acting *via* the proposed surfactant AOP and can be used to assess chemicals within the Surfactant Category.

For general cytotoxicity ([REF _Ref46931271 \h * MERGEFORMAT]), cell lines are available that are known to be sensitive to the effects of surfactants. The BALB/c 3T3 NRU cytotoxicity test has been reviewed and recommended by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) for use prior to conducting animal testing [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The surfactants with known inhalation toxicity (*e.g.,* octylphenoxypolyethoxyethanol, oleoyl sarcosine, DDAC, or BAC) should be tested in parallel with the new chemical substance to benchmark the results, thereby providing reliable results for estimating the potential for surfactants to cause irritation and cytotoxicity.

OLEs

Based on the results of the testing on the CLEs, given the limitations of the assays, it may be necessary to perform more robust testing. The discussed assays measure single cell types, whereas human and animal airway epithelia are composed of multiple cell types that each have specialized functions. Several human airway models have been developed that allow for the assessment of multiple endpoints in 3D culture systems. Two commonly employed systems are EpiAirway™ and MucilAir™ developed by MatTek Life Sciences and Epithelix, respectively.

Organotypic airway epithelial cultures, such as EpiAirway™ and MucilAir™ are more physiologically relevant than *in vitro* cell lines [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2018</Year><RecNum>14811</RecNum><DisplayText>[107]</DisplayText><record><rec-number>14811</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr"

timestamp="1596045320">14811</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Issue Paper: Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity: A Case Study Using a New Approach Methodology (NAM)</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>33, https://ntp.niehs.nih.gov/ntp/about_ntp/sacatm/2019/september/bcgnd-1-epa_case_study.pdf</pages><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>]. These organotypic cultures, unlike single cell lines, take on a pseudostratified morphology; develop tight junctions; differentiate into multiple cell types, including basal cells, ciliated cells, and goblet cells; generate mucus; exhibit ciliary beating; have xenobiotic metabolizing capacity; and maintain cultural homeostasis for months. Because of these characteristics, these human airway models are expected to better represent the response of *in vivo* tissue to surfactant exposure than cell line cultures of a single cell type. Depending on the anatomical area in the respiratory system where the site of contact/exposure is predicted to occur, using for example RDDR or multi-path particle dosimetry (MPPD) modeling for determining deposition, different 3D cell culture systems are available that are composed of the different cell types that occur at different anatomical sites in the respiratory tract. MucilAir™ provides 3D co-culture models of cells from nasal, tracheal or bronchial sites, as well as a co-culture of cells from small airways (SmallAir™). EpiAirway™ is composed of a co-culture of normal human

tracheal/bronchial epithelial cells, and EpiAlveolar™ is a 3D co-culture model of the air-blood barrier produced from primary human alveolar epithelial cells, pulmonary endothelial cells, and fibroblasts (with and without macrophages).

Exposure of respiratory tract 3D co-culture models to aerosols at the air liquid interface (ALI) using an *in vitro* exposure system, such as those available from Vitrocell® Systems, provides an exposure more comparable to real-life scenarios for inhaled aerosols, although it is a lower throughput compared to *in vitro* two-dimensional exposure systems. Dilution in medium and interaction with medium components does not occur in the ALI exposure systems as in submerged culture systems. The respiratory tract 3D co-culture models are more physiologically relevant due to the fact there is an interaction of the aerosol with a mucus or surfactant layer, as as in humans.

Exposures of these organotypic cultures at the ALI can be combined with other assays for assessing cell function and viability to inform the surfactant AOP elements. Measurement of transepithelial electrical resistance (TEER), LDH-release, and viability assays such as MTT or ATP assays have all been reported for use with these cultures. Further, multiple assays can be performed on the same cultures. TEER measures epithelial integrity, including functionality of intercellular tight junctions. LDH-release measures loss of plasma membrane integrity, which is indicative of cytotoxicity, and MTT and ATP assays measure cell viability. MatTek Life Sciences recommends the MTT assay for use with their EpiAirway™ cultures and recommends the surfactant octylphenoxypolyethoxyethanol at 0.2% concentration as a positive control for cytotoxicity. These assays can also be used to determine an HEC, provided dosimetry models are

available for translation of the internal dose achieved under culture conditions to an equivalent inhalation exposure for the human scenario of interest. Examples of *in vitro* dosimetry models to predict particle doses for submerged cell culture include the *In vitro* Sedimentation, Diffusion and Dosimetry model (ISDD) [ADDIN EN.CITE ADDIN EN.CITE.DATA] and the *In vitro* Sedimentation, Diffusion and Dissolution Dosimetry (ISD3) model [ADDIN EN.CITE ADDIN EN.CITE.DATA].

Significant progress has been made toward achieving the objectives to use high-throughput *in vitro* assays and computational models to evaluate potential adverse effects of chemical exposures [ADDIN EN.CITE <EndNote><Cite><Author>NRC</Author><Year>2007</Year><RecNum>14741</RecNum><DisplayText>[14, 110]</DisplayText><record><rec-number>14741</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019531">14741</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>NRC</author></authors></contributors><titles><title>Toxicity Testing in the 21st Century: A Vision and a Strategy, Washington, D.C. The National Academies Press</title></titles><pages>216, DOI: https://doi.org/10.17226/11970</pages><volume>ISBNs: Ebook: 978-0-309-13412-5; Paperback: 978-0-309-15173-3</volume><dates><year>2007</year></dates><urls></urls></record></Cite><Cite><Author>NRC</Author><Year>2017</Year><RecNum>14812</RecNum><record><rec-number>14812</rec-number><foreign-keys><key app="EN" db-

id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596045703">14812</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>NRC</author></authors></contributors><titles><title>Using 21st Century Science to Improve Risk-Related Evaluations, Washington, D.C., The National Academies Press</title></titles><pages>200, https://doi.org/10.17226/24635</pages><volume>ISBNs: Ebook: 978-0-309-45351-6; Paperback: 978-0-309-45348-6</volume><dates><year>2017</year></dates><urls></urls></record></Cite></EndNote>]. To translate the effects to higher levels of biological organization, a battery of assays with varying complexity and physiological relevance may be needed. The 3D human airway cell culture systems are available to add evidence to the AOP and increase confidence of the physiological relevance to humans.

Precision-cut lung slices (PCLS) is an additional method to develop OLE data. The PCLS measures multiple endpoints, such as LDH for cytotoxicity and IL-1α for pro-inflammatory cytokine release, in *ex vivo* cultures of rodent and human lung slices, to determine whether a chemical is likely to be toxic to the respiratory tract by inhalation exposure [ADDIN EN.CITE ADDIN EN.CITE.DATA]. PCLS contain intact alveoli, rather than monolayers of one or two cells types (co-cultures). Crucially, in contrast to organoids, cell types are present in the same ratios and with the same cell–cell and cell–matrix interactions as *in vivo* systems. PCLS are often used in toxicological and anatomical studies regarding contractility in relation to asthma and other respiratory illnesses, such as emphysema [ADDIN EN.CITE <EndNote><Cite><Author>Sanderson</Author><Year>2011</Year><RecNum>14814</RecN

um><DisplayText>[112]</DisplayText><record><rec-number>14814</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046031">14814</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sanderson, M. J.</author></authors></contributors><auth-address>Department of Microbiology and Physiological Systems, University of Massachusetts Medical School, Worcester, MA 01655, USA. Michael.Sanderson@umassmed.edu</auth-address><titles><title>Exploring lung physiology in health and disease with lung slices</title><secondary-title>Pulm Pharmacol Ther</secondary-title><alt-title>Pulmonary pharmacology & therapeutics</alt-title></titles><periodical><full-title>Pulmonary pharmacology & therapeutics</full-title><abbr-1>Pulm Pharmacol Ther</abbr-1></periodical><alt-periodical><full-title>Pulmonary pharmacology & therapeutics</full-title><abbr-1>Pulm Pharmacol Ther</abbr-1></alt-periodical><pages>452-65</pages><volume>24</volume><number>5</number><edition>2011/05/24</edition><keywords><keyword>Animals</keyword><keyword>Cell Physiological Phenomena</keyword><keyword>Disease Models, Animal</keyword><keyword>Humans</keyword><keyword>Lung/pathology/*physiology</keyword><keyword>Lung Diseases/*pathology</keyword><keyword>Microscopy/methods</keyword><keyword>Muscle Contraction/physiology</keyword><keyword>Organ Culture Techniques</keyword></keywords><dates><year>2011</year><pub-dates><date>Oct</date></pub-dates></dates><isbn>1094-5539 (Print)
1094-5539</isbn><accession-num>21600999</accession-

num><urls></urls><custom2>PMC3168687</custom2><custom6>NIHMS296121</custom6><electronic-resource-num>10.1016/j.pupt.2011.05.001</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]. Therefore, physiological responses, other than cytotoxicity, that may be evoked by the surfactant may be evaluated. One further advantage of PCLS is that the assay can be performed on multiple species to determine inter-species variability in susceptibility.

The PCLS test system has been pre-validated in multiple, independent laboratories, and the results showed correlation with *in vivo* LC_{50} values [ADDIN EN.CITE ADDIN EN.CITE.DATA]. While considered an alternative test, use of rodent tissue still requires use of animals, but when compared to *in vivo* inhalation tests, this assay reduces the number of animals that would be needed to conduct dose response studies. From a rat lung (1 g), approximately 200 slices can be prepared. In general, for each test substance concentration, 2 slices are used, resulting in 100 different concentrations or repeats that can be tested using tissue from a single rat. Additionally, PCLS cultures are stable for up to 4 weeks and allows for exposures *via* liquid media or, with additional adaptations, air. As such, the PCLS system meets the goal of reducing animal testing, although dosimetry models for their translation to HEC are not yet developed. Human PCLS, derived from, for example, rejected but otherwise healthy transplant tissue, can also be used to measure cell/tissue viability, local respiratory inflammation and physiological function. These endpoints can be measured in single and repeated exposures in a metabolically competent system within the normal architecture of the lung in a more relevant model system, replacing the need for animal testing [ADDIN EN.CITE ADDIN

EN.CITE.DATA]. Mechanistic rodent and human PCLS studies may be conducted in parallel to understand species specific difference in toxicological effects. The rationale for selection of the PCLS assay, as with any inhalation toxicity assay, should be scientifically justified in advance of initiating testing.

Uncertainties/Limitations of the Surfactant AOP Approach

A number of *in vitro* assays have been discussed as to their potential utility within the context of surfactant AOP elements (*i.e.*, MIEs, CLEs, and OLEs). Uncertainties and limitations associated with these assays are discussed for each of the above testing systems, as well as others [ADDIN EN.CITE ADDIN EN.CITE.DATA], it is important to consider that these assays were not systematically tested using surfactants or benchmarked against *in vivo* inhalation toxicity data on surfactants using traditional test method validation approaches. Nonetheless, these assays, alone or in combination should be considered to provide information on whether a new chemical meets the Surfactant Category criteria and/or to understand whether the new chemical may be more or less bioactive or toxic than the sub-category comparator chemicals. EPA will generally use the framework and analogue toxicity data identified in this investigation to assess potential risks from surfactants.

In this regard, approaches to evaluate the scientific confidence of test methods for hazard assessment and risk assessment continues to evolve. A fit-for-purpose framework, employing specific criteria to establish relevancy, reliability, variability, sensitivity, and domain of applicability for evaluating a new method to inform specific decisions has emerged from the regulatory science community to address the challenges posed for validation of NAMs [ADDIN

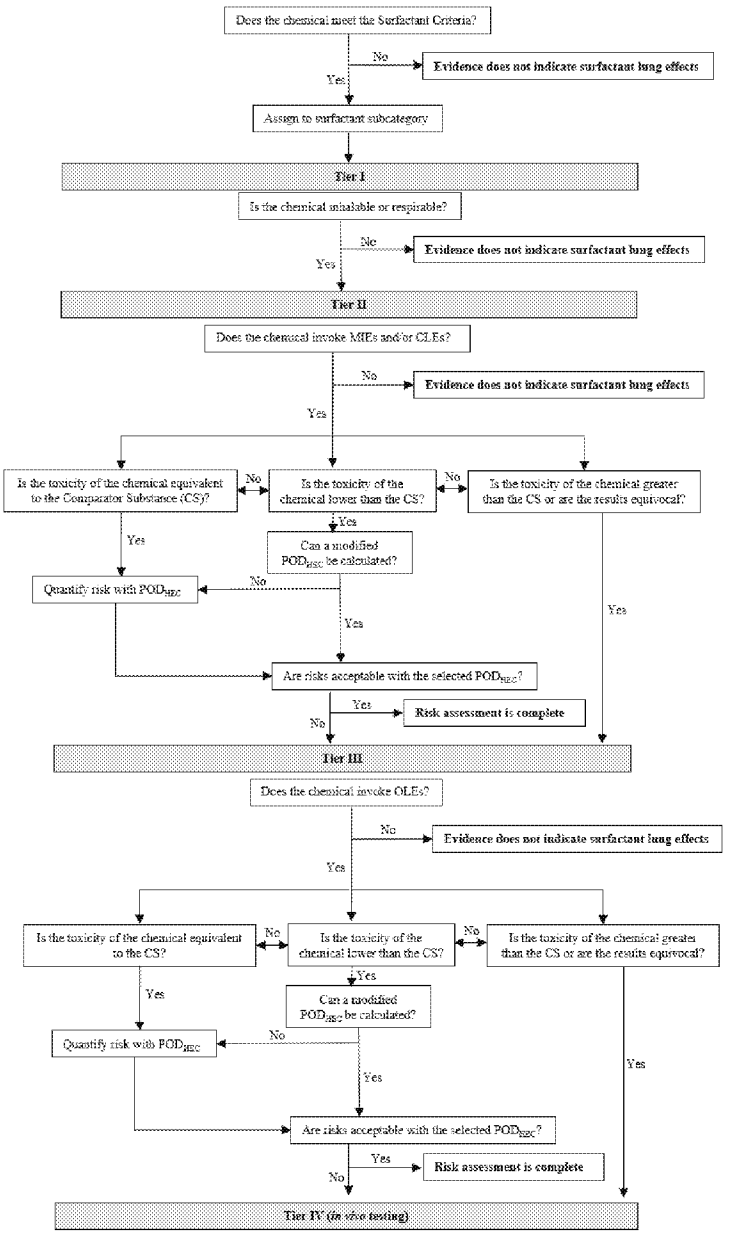
EN.CITE ADDIN EN.CITE.DATA]. Such fit-for-purpose validation approaches are intended to be flexible and adaptable and to provide data sets, prediction analysis results, inference models, *etc*. in a transparent manner that enable other scientists to confirm the performance of the assays and inference models, as well as evaluate the rationale for using these assays in a specific decision context.

Once such fit-for-purpose scientific evaluations are documented, there are several ways that these assays can be used to reduce and replace animal testing. First, testing can be performed based on the surfactant AOP to evaluate the potency of new surfactants versus a comparator substance within the relevant subcategory that has repeated exposure inhalation toxicity data. Second, depositional data using models such as RDDR or MPPD for determining the depositional fraction of the new surfactant may be used for test concentration estimation and for estimating a potency ratio. Finally, *in vitro* to *in vivo* extrapolations (IVIVEs) may be used to determine a HEC for quantitative risk assessment.

Tiered-testing Strategy

The first step in the tiered-testing strategy is to determine if the evaluated substance meets the Surfactant Criteria. If so, then assign the substance to the appropriate surfactant subcategory (nonionic, anionic, or cationic) and determine whether any of the representative subcategory chemicals may serve as an acceptable toxicological analogue for risk assessment or as a comparator substance for tiered testing. If a representative subcategory chemical is determined to be an acceptable toxicological analogue to the new chemical substance, then quantify risks using the toxicological analogue. If the MOE is equal to or greater than the benchmark MOE, then

tiered testing is not required on the new chemical substance. If the MOE is lower than the benchmark MOE or if a determination cannot be made on whether any of the representative subcategory chemicals are acceptable toxicological analogues, then proceed with tiered testing using the most appropriate subcategory chemical as a comparator substance to the new chemical substance. As detailed below, the tiered-testing strategy commences with the least complex, most efficient testing methods, and at each subsequent tier, the complexity of the test system increases, commensurate with the hypothesized surfactant AOP, to more effectively emulate the biology and physiology of the *in vivo* respiratory tract system. It is envisioned that both the new chemical substance and the comparator substance will be evaluated side-by-side in the NAM assays. The results of these studies may lead to the conclusion that the comparator substance is an acceptable toxicological analogue to the new chemical substance. Alternatively, the results may support that higher tiered testing is warranted, particularly when the new chemical substance has ~~lower or~~ higher toxicity than the comparator substance. If *in vivo* testing is conducted, it may not be necessary to run the comparator substance in the *in vivo* tests, given that suitable inhalation studies are available on the comparator substances. A summary of the proposed tiered-testing strategy is provided in [REF _Ref48210489 \h * MERGEFORMAT] and discussed further below.



Scheme **[SEQ Scheme * ARABIC]**. Proposed tiered-testing strategy for general surfactants.

Tier I—Physicochemical properties

Surfactants are proposed to cause a specific sequence of biological events in the respiratory tract if they are inhaled. Manufacture, processing, or use of a surfactant in an inhalable form, (*i.e.*, ≤ 100 μm aerodynamic diameter) is therefore, an initial consideration of the potential for a surfactant to cause toxicity to the respiratory tract. Particle size is an established parameter for determining inhalability/respirability of particles/droplets. Several validated test methods exist for determining potential inhalability/respirability, *i.e.*, particle size, of a new chemical substance (*e.g.*, OECD GD 39 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>14819</RecNum><DisplayText>[77]</DisplayText><record><rec-number>14819</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046851">14819</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Guidance Document on Inhalation Toxicity Studies, Series on Testing and Assessment, No. 39 (Second Edition)</title><secondary-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</secondary-title></titles><periodical><full-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</full-title></periodical><pages>106, https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2009)2

8/rev1&doclanguage=en</pages><volume>ENV/JM/MONO(2009)28/REV1</volume><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>], ISO 21501-1:2009 [ADDIN EN.CITE <EndNote><Cite><Author>ISO</Author><Year>2009</Year><RecNum>14820</RecNum><DisplayText>[116]</DisplayText><record><rec-number>14820</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046993">14820</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>ISO</author></authors></contributors><titles><title>Determination of particle size distribution — Single particle light interaction methods — Part 1: Light scattering aerosol spectrometer</title></titles><pages>https://www.iso.org/standard/42728.html</pages><volume>ISO 21501-1:2009</volume><dates><year>2009</year></dates><urls></urls></record></Cite></EndNote>], OECD TG 110 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>1981</Year><RecNum>14821</RecNum><DisplayText>[117]</DisplayText><record><rec-number>14821</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596047114">14821</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Particle Size Distribution/Fibre Length and Diameter Distributions; Method A: Particle Size Distribution (effective hydrodynamic radius); Method B: Fibre Length and Diameter

Distributions</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>13, https://www.oecd-ilibrary.org/docserver/9789264069688-en.pdf?expires=1596047951&id=id&accname=guest&checksum=A9C13F0DFDCF2A5DD4DD39DAC64C47BC</pages><volume>110</volume><dates><year>1981</year></dates><urls></urls></record></Cite></EndNote>], and OPPTS 830.7520 [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1996</Year><RecNum>14822</RecNum><DisplayText>[118]</DisplayText><record><rec-number>14822</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596047315">14822</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Particle Size, Fiber Length, and Diameter Distribution</title><secondary-title>Product Properties Test Guideline, Office of Pollution Prevention and Toxics, U.S. Enviornmental Protection Agency</secondary-title></titles><periodical><full-title>Product Properties Test Guideline, Office of Pollution Prevention and Toxics, U.S. Enviornmental Protection Agency</full-title></periodical><pages>13, https://www.regulations.gov/contentStreamer?documentId=EPA-HQ-OPPT-2009-0151-0030&contentType=pdf</pages><volume>EPA 712-C-96-037</volume><dates><year>1996</year></dates><urls></urls></record></Cite></EndNote>]). The studies shown in Table 3 suggest that the total respiratory tract may be affected from surfactants; therefore, inhalable forms ($\leq$ 100 μm) were identified as the most relevant for quantitative inhalation risk assessment. As a practical matter, a particle size cutoff of greater than

1% inhalable particles/droplets by weight (wt%), determined in a well conducted study using a valid measurement method will generally be considered as triggering a quantitative assessment of inhalation toxicity on a new chemical substance meeting the Surfactant Criteria. EPA will generally assess the potential inhalation toxicity for a new surfactant chemical substance when the manufacture, processing or use results in greater than 1% (by weight) of the surfactant particles/droplets having a particle size of less than 100 µm. This wt% cutoff is consistent with EPA's "trace amounts" threshold for the nonreportable content for nanoscale materials [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2017</Year><RecNum>14823</RecNum><DisplayText>[119]</DisplayText><record><rec-number>14823</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596047488">14823</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Chemical Substances When Manufactured or Processed as Nanoscale Materials; TSCA Reporting and Recordkeeping Requirements</title><secondary-title>Federal Register</secondary-title></titles><periodical><full-title>Federal Register</full-title></periodical><pages>3641-3655</pages><volume>82</volume><number>8</number><dates><year>2017</year></dates><urls></urls></record></Cite></EndNote>].

If inhalable particles/droplets can be generated at greater than 1 wt% during manufacturing, processing, or any of the uses for the new chemical substance, proceed to Tier II.

Tier II—*In vitro/Ex vivo* studies

The following *in vitro/ex vivo* test methods may provide potentially useful information to determine whether a new chemical substance invokes MIEs and CLEs. In order to determine the best approach for *in vitro/ex vivo* testing, a pre-notice consultation with EPA is highly encouraged ~~given that, for surfactants, none of the following studies have been validated using the traditional interlaboratory round robin method to determine lung effects/toxicity~~. In general, the testing approach in this tier should include a combination of assays, such as one that measures MIE #1 (*e.g.*, epithelial lining fluid/cell perturbation or pulmonary surfactant interaction/loss of function), one that measures MIE #2 (*e.g.*, cell membrane disruption/interaction/penetration), and one that measures CLEs (*e.g.*, loss of membrane integrity/general cytotoxicity) (see [REF _Ref46931271 \h * MERGEFORMAT]). *In vitro/ex vivo* eye irritation studies may also demonstrate cell interaction or penetration and general cytotoxicity.

For each assay, the comparator substance for the respective subcategory of surfactants should be tested under identical conditions. Further, the particle size distribution data may be used with dosimetry models such as RDDR model or the MPPD model to aid with identifying the regions in the respiratory tract where deposition is expected to occur and the appropriate test concentrations for the *in vitro/ex vivo* test systems, considering for example the surface area of the culture system or *ex vivo* tissue, loss mechanisms, *etc.*

Notwithstanding the uncertainties with the above assays, each may be used to determine a starting point to calculate a modified POD_{HEC} using *in vitro* to *in vivo* extrapolation (IVIVE) for

the purpose of evaluating the relative potency of the new chemical substance versus the comparator substance. Several investigations have provided insight on approaches for accomplishing this, although with different assay systems [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In doing so, a weight of scientific evidence evaluation should be performed considering the structural features, physicochemical properties, and assay results on the new chemical substance versus the comparator substance. Based on this evaluation, the most biologically relevant endpoint(s) should be used to calculate a POD. BMD modeling may be applied to derive a $BMCL_{1SD}$ metric, as a possible metric, although the metric of one standard deviation should be used with caution [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2019</Year><RecNum>14825</RecNum><DisplayText>[121]</DisplayText><record><rec-number>14825</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596048386">14825</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Transmittal of Meeting Minutes and Final Report for the Federal Insecticide Fungicide and Rodenticide Act, Science Advisory Panel (FIFRA SAP) Meeting held on December 4 and 6, 2018</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>51,https://www.regulations.gov/contentStreamer?documentId=EPA-HQ-OPP-2018-0517-0030&contentType=pdf</pages><volume>EPA-HQ-OPP-2018-

0517</volume><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>]. Alternative metrics should be considered, as our understanding evolves for various *in vitro* assays and endpoints. For example, the pharmaceutical industry has used fixed adverse response thresholds that are appropriate for the specific biological assay (*i.e.*, EC_{15}, EC_{30}, *etc.*) [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Regardless of the metric used, a justification for its selection should be provided. In those situations where data are not amenable to BMD modeling, the *in vitro* concentration tested should be determined based on the expected HEC for the appropriate subcategory (taking into account the necessary MOE) to ensure that the *in vitro* data are generated in a concentration range relevant to the expected HEC.

Given that the understanding of IVIVE is evolving, assay results should be interpreted in a manner consistent with the weight of scientific evidence, as noted above, while recognizing that uncertainties are often dealt with by erring on the side of conservativism. Therefore, the following initial default criteria are proposed for utilizing the assay results, and when possible, the IVIVE estimates. These criteria are consistent with EPA's approach for evaluating non-vertebrate animal skin sensitization data [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2018</Year><RecNum>14832</RecNum><DisplayText>[123]</DisplayText><record><rec-number>14832</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596244984">14832</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Interim Science Policy: Use of Alternative Approaches for Skin Sensitization as a Replacement

for Laboratory Animal Testing (draft for public comment: April 4, 2018)</title><secondary-title>Office of Chemical Safety and Pollution Prevention & Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention & Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>13, https://www.regulations.gov/contentStreamer?documentId=EPA-HQ-OPP-2016-0093-0090&contentType=pdf</pages><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>], while recognizing that the weight of scientific evidence may support an alternative interpretation to the default criteria.

The Tier II assays evaluate biologically relevant endpoints representing events in the hypothesized surfactant AOP. The results of the comparator substance and the new chemical substance in these assays provide a basis for evaluating the suitability of using the comparator substance to evaluate toxicity ~~to~~ of the new chemical substance.

If comparable toxicity is observed between the comparator substance and the new chemical substance in the Tier II assays, the POD_{HEC} from the comparator substance may be appropriately used as a toxicological analogue for quantifying the MOE. If calculated risk is acceptable stop at Tier II, otherwise proceed to Tier III.

If lower toxicity is observed for the new chemical substance versus the comparator substance in the Tier II assays, then these data should be used to determine if a modified POD_{HEC} can be

quantified for the new chemical substance. If this is possible, the modified POD_{HEC} for the new chemical substance should be used for quantifying the MOE. If calculated risk is acceptable, then stop at Tier II. However, if it is not possible to calculate a modified POD_{HEC}, then the comparator substance POD_{HEC} could be used as a worse-case toxicological analogue for risk assessment. If no acceptable risk can be calculated, proceed to Tier III.

If greater toxicity is observed with the new chemical substance versus the comparator substance in the Tier II assays, suggesting risks would be identified as unacceptable, proceed to Tier III. Alternatively, there may be scientifically justified reasons for an alternative interpretation, which should be clearly articulated with the weight of scientific evidence evaluation. Otherwise, it may be necessary to proceed to Tier III.

Commented [HT28]: Changed a bit for clarity

If the results from the Tier II assays are equivocal (*i.e.*, they do not demonstrate comparable or lower toxicity of the new chemical substance versus the comparator substance), and there is no clear rationale or explanation, then proceed to Tier III testing because the data are too uncertain to make a reasoned evaluation on the potential health risks, following potential inhalation exposures.

Tier III – 3D Human Airway Models/PCLS Assay

Several testing options are available for evaluating OLEs in the surfactant AOP. The test system employed should focus on evaluating effects in the respiratory tract at the predicted sites of deposition (*e.g.*, ET, TB and/or PU regions), based on the particle size distribution data

generated under Tier I and using RDDR or MPPD modeling. A justification for using a particular system(s) should be provided and may be discussed with EPA as part of a pre-notice consultation. Representative test systems include those listed in [REF _Ref46931271 \h * MERGEFORMAT].

Based on the results of the 3D-construct and/or PCLS testing, IVIVE may be possible for developing a POD_{HEC} for use with characterizing potential risks using the MOE approach. Though the occupational/consumer exposure estimates may be the same between Tiers II and III, the Tier III test results may offer the opportunity for refining the risk estimates. For example, the BMR used for calculating the POD_{HEC} may be refined because the ALI-based exposure is more consistent with inhalation exposure in a human than the submerged culture exposures employed in Tier II [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2018</Year><RecNum>14811</RecNum><DisplayText>[107]</DisplayText><record><rec-number>14811</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596045320">14811</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Issue Paper: Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity: A Case Study Using a New Approach Methodology (NAM)</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S.

Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>33, https://ntp.niehs.nih.gov/ntp/about_ntp/sacatm/2019/september/bcgnd-1-epa_case_study.pdf</pages><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>]. Further, application of uncertainty factors for calculating the benchmark MOE may also be refined, if for example, human cultures are used, which may preclude the need for applying a UF_A.

If the Tier III test data are amenable for developing a POD_{HEC}, then the risk estimates should be reassessed. If no risks are identified under the conditions of use, then stop at Tier III. If risks are still identified under the conditions of use or if the Tier III test data are not amenable for developing a POD_{HEC}, then proceed to Tier IV.

Tier IV – *In vivo* studies

Strategic *in vivo* testing may be ~~needed~~ considered as a last resort to inform the hazard and risk assessment of new chemical substances, particularly in those instances where a new chemical substance has unique properties that preclude a determination that one of the comparator substances in a subcategory has representative toxicological properties to the new chemical substance, as well as in instances where the test data generated under Tiers II and III are not amenable for deriving modified POD_{HEC}s. ~~If *in vivo* testing is needed, a~~A pre-notice consultation meeting with EPA is strongly encouraged prior to initiating any vertebrate animal testing. This point is especially important because TSCA section 4(h)(3) indicates that any person developing information for submission under TSCA section 5 on a voluntary basis shall first attempt to develop the information by means of an alternative test method or strategy identified by EPA

before conducting new vertebrate animal testing [ADDIN EN.CITE <EndNote><Cite><Author>U.S.C.</Author><Year>2016</Year><RecNum>14796</RecNum><DisplayText>[83]</DisplayText><record><rec-number>14796</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041048">14796</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>U.S.C.</author></authors></contributors><titles><title>Title 15-Commerce and Trade, Chapter 53-Toxic Substances Control, Subchapter I-Control of Toxic Substances</title><secondary-title>United States Code (U.S.C.)</secondary-title></titles><periodical><full-title>United States Code (U.S.C.)</full-title></periodical><pages>https://uscode.house.gov/view.xhtml?path=/prelim@title15/chapter53&edition=prelim</pages><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>].

The potential for surfactants to cause adverse effects on the respiratory tract are based on acute toxicity concerns, that is, interfering with epithelial lining fluid/pulmonary surfactant and/or disrupting cellular membranes and epithelial cytotoxicity. Since these effects may be captured using appropriate exposure concentrations in short-term inhalation studies, the following *in vivo* tests should be considered:

- Step 1: OECD Acute TG 403 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2009</Year><RecNum>14827</RecNum><DisplayText>[124]</DisplayText><record><rec-number>14827</rec-

Commented [ST29]: Should we replace this with 436, since it uses fewer animals?

number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596048858">14827</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Acute Inhalation Toxicity</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>19, https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd-tg403.pdf</pages><volume>403</volume><dates><year>2009</year></dates><urls></urls></record></Cite></EndNote>] (modified)** [illegible] rats exposed for 4 hours and observed for 2 weeks using aerosol exposure.

- Step 2: 5-Day inhalation study with a 14-day observation period** to address progression/resolution of effects. The OECD TG 412 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>14828</RecNum><DisplayText>[125]</DisplayText><record><rec-number>14828</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596048957">14828</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>28-day (subacute) inhalation toxicity study</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>23,

https://doi.org/10.1787/9789264070783-en</pages><volume>412</volume><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>] should be used, but the exposure duration should be 5 days.

**Modifications to the above studies should be discussed with EPA during a pre-notice consultation meeting and may include pulmonary function testing (if measurable), analysis of BALF, LDH release, complete histopathological analysis of the respiratory tract and blood oxygen (pO_2) content. OECD TG 412 and OECD GD 39 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>14819</RecNum><DisplayText>[77]</DisplayText><record><rec-number>14819</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046851">14819</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Guidance Document on Inhalation Toxicity Studies, Series on Testing and Assessment, No. 39 (Second Edition)</title><secondary-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</secondary-title></titles><periodical><full-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</full-title></periodical><pages>106, https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2009)28/rev1&doclanguage=en</pages><volume>ENV/JM/MONO(2009)28/REV1</volume><d

ates><year>2018</year></dates><urls></urls></record></Cite></EndNote>] should be consulted. Additionally, the sensory irritant potential can be measured using ASTM E 981 to determine reflex inhibition [ADDIN EN.CITE <EndNote><Cite><Author>Alarie</Author><Year>2001</Year><RecNum>14826</RecNum><DisplayText>[126]</DisplayText><record><rec-number>14826</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596048712">14826</key></foreign-keys><ref-type name="Book Section">5</ref-type><contributors><authors><author>Alarie, Y.</author><author>Nielsen, G.D.</author><author>Schaper, M.M.</author></authors><secondary-authors><author>Spengler, B.</author><author>Samet, J. M.</author><author>McCarthy, J.F.</author></secondary-authors></contributors><titles><title>Animal Bioassays for Evaluation of Indoor Air Quality</title><secondary-title>Indoor Air Quality Handbook</secondary-title></titles><pages>23.21-23.49.</pages><dates><year>2001</year></dates><pub-location>New York</pub-location><publisher>McGraw-Hill</publisher><urls></urls></record></Cite></EndNote>].

The results of the *in vivo* testing should be used for reassessing and recharacterizing the risks of the new chemical substance.

CONCLUSIONS

The overall objective of this investigation was to develop a chemical category for use in conducting inhalation risk assessment for new chemical surfactant substances under TSCA. This

investigation developed physical-chemical properties, *i.e.*, the Surfactant Criteria, assessors and product stewards can use for determining whether a new chemical substance can be considered a surfactant. Further, properties and characteristics are provided to divide the Surfactant Category into sub-categories for nonionic, anionic, and cationic surfactants, which is important from a toxicological perspective. A systematic literature search and review were conducted to identify data to define a Surfactant Category and substances from which PODs were identified from inhalation toxicity studies. To facilitate chemical comparisons, animal toxicity studies that could be used to derive PODs for risk assessments were identified for at least one chemical substance for each sub-category and converted to HECs using established methods developed by EPA. Finally, a tiered-testing strategy for generating *de novo* data for new surfactant substances is provided that integrates a variety of currently available NAMs using a hypothesized AOP framework. ~~Though the tiered-testing strategy may be aspirational for a variety of reasons (e.g., evolving understanding of the representativeness of *in vitro* systems to *in vivo* systems, jurisdictional requirements for vertebrate animal testing, uncertainty associated with the comparability of the new chemical substance to the comparator substance is so great that testing is needed, etc.), the~~ The use of this tiered-testing strategy will inform the available data on surfactants and provide greater confidence in the use of non-vertebrate testing approaches for assessing the potential risks of new chemical substances. It also offers advantages to regulators, the regulated community, and consumers because: 1) integrating NAMs into a category testing approach supports EPA, TSCA and product stewardship goals of reducing and replacing vertebrate animal testing; 2) decision analysis for higher tiered testing takes into consideration mechanistic responses, dosimetry and exposure information , and 3) it encourages development

of mechanistic data to advance the understanding of the potential inhalation toxicity of surfactants, which will drive the development of newer and safer chemistries.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information file contains the following:

Section 1. Systematic Literature Review

Section 2. RDDR Modeling Outputs

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally.

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Notes

Disclaimer: The views expressed in this article are those of the authors and do not necessarily represent the views or policies of their respective employers. Mention of trade names or commercial products does not constitute endorsement for use.

Disclosures: TS, AMJ, KS, WI, and TRH are employed by the federal government. MPH, WK, AMK, SM, LJ, JLR, AT, and RT are employed by companies that manufacture, process, and/or use surfactants. RAB and SOS are employed by a company that represents companies that manufacture, process, and/or use surfactants. PDM and SDS work for a company that received contract funding from companies that manufacture, process, and/or use surfactants. MO and JM work for a company that receives contract funding from the federal government. AJC and MS are employed by a company whose mission is to advance animal-free testing approaches that protect human health and the environment

REFERENCES

[ADDIN EN.REFLIST]

Message

From: Stedeford, Todd [Stedeford.Todd@epa.gov]
Sent: 8/13/2020 3:37:59 PM
To: Sahar_Osman-Sypher@americanchemistry.com
CC: Henry, Tala [Henry.Tala@epa.gov]; Salazar, Keith [Salazar.Keith@epa.gov]; Irwin, William [Irwin.William@epa.gov]
Subject: Surfactants --> revised draft 13 August.ver.2
Attachments: draft manscript general surfactants - 13 August 2020.ver.2.docx; Tiered-testing scheme for surfactants - 13 August 2020.ver.1.pptx

Importance: High

Hi Sahar,

Here is the revised draft and the PowerPoint for Keith's Schematic. I included the PPT incase any typos are spotted; the schematic is inserted into the manuscript. In addition to numerous editorial items, Tala also re-wrote the paragraph on literature review under the results and discussion, after going through the supporting information. Just one comment for Stefan and a question for Jane, shown below. If possible, I'd like to get everyone's comments by COB today.

Thanks,

Todd

Ex. 5 Deliberative Process (DP)

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Surfactants Category: The Application of a New Approach Methodology (NAM) for Assessing Inhalation Risks under the Amended Toxic Substances Control Act

Commented [HT1]: NOTE: EPA's Strategy AND the Tiered Testing Strat in the paper uses multiple assays, so this should be plural

BUT this is now different from the LO paper, so keep or change?

Commented [ST2R1]: The idea is that the tiered-testing strategy is the NAM, not the individual assays

Tala R. Henry[a,‡], *Keith D. Salazar*[b,‡], *Michael P. Hayes*[c], *Wayne Kennedy*[d], *Athena M. Keene*[d], *Annie M. Jarabek*[e], *Stefan Moors*[f], *Lela Jovanovich*[g], *Jane L. Rose*[c], *Ann Tveit*[f], *Raphaël T. Tremblay*[c], *Richard A. Becker*[h], *Sahar Osman-Sypher*[h], *Patrick D. McMullen*[i], *Scott D. Slattery*[j], *William Irwin*[b], *Marc Odin*[j], *Julie Melia*[j], *Monita Sharma*[k], *Amy J. Clippinger*[k], *and Todd Stedeford*[a,*]

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KEYWORDS: Inhalation, Surfactant, New Approach Methodologies, Lung Toxicity, Risk Assessment

Commented [HT3]: NEW

ABSTRACT

The Toxic Substances Control Act (TSCA) requires anyone who plans to manufacture (including import) a new chemical substance for a non-exempt commercial purpose to provide the U.S. Environmental Protection Agency (EPA) with a premanufacture notice (PMN) prior to commercialization. Surfactants are a class of chemical substances used in a variety of industrial operations, occupational settings, and in consumer products. Their uses in such applications provide pathways of exposure by which potential toxicity of these compounds may occur to humans. While TSCA requires submission of any existing toxicity data, it does not require generation of toxicity data for the purpose of, or prior to, submitting a PMN. TSCA requires

EPA to review the PMN to determine whether the new chemical substance presents an unreasonable risk of injury to human health or the environment and mandates that EPA reduce or replace vertebrate animals in testing, to the extent practicable and scientifically justified. EPA therefore relies on several approaches that do not rely on *de novo* toxicity testing. Analogue read-across, in which toxicity data for a chemical of similar structure and activity are used to assess the new chemical, and chemical categories (a group of chemicals whose properties are likely to be similar or follow a regular pattern as a result of mechanism, mode of toxic action or structural similarity) have been used by EPA for decades to assess new chemical substances. This investigation was conducted to identify surfactant chemicals with toxicity data relevant for use in conducting a quantitative human health risk assessment for new surfactant substances and to define a TSCA New Chemical Category for surfactants. Category boundaries, which are defined, toxicological analogues suitable for conducting 'read-across' hazard assessment (*i.e.*, hazard identification and dose-response analysis) are identified and a tiered-testing strategy aimed at using new approach methodologies (NAMs) to reduce or replace animal testing is outlined. This tiered strategy to defining and evaluating the Surfactant Category provides a pragmatic and scientifically defensible approach to facilitate EPA's review of PMNs for new surfactants and a strategic testing approach that provides the data needed to conduct or refine surfactant risk assessments while also meeting the requirements of TSCA to reduce vertebrate testing.

Commented [SK4]: Capitalization was inconsistent throughout. I capitalized everywhere.

INTRODUCTION

The Toxic Substances Control Act (TSCA) was amended in 2016 by the Frank R. Lautenberg Chemical Safety for the 21st Century Act (Pub. L. 114-182). The amended TSCA included substantial changes to EPA's authorities and responsibilities, including requirements on EPA to

make a determination regarding sufficiency of information, environmental releases and human exposure, and unreasonable risks. The amended TSCA also included provisions mandating EPA to "reduce and replace, to the extent practicable, [and] scientifically justified" the use of vertebrate animals in the testing of chemicals substances. Specifically, TSCA section 4(h) charges EPA with encouraging and facilitating –

> (1) the use of scientifically valid test methods and strategies that reduce or replace the use of vertebrate animals while providing information of equivalent or better scientific quality and relevance that will support regulatory decisions under TSCA;
> (2) the grouping of 2 or more chemical substances into scientifically appropriate categories in cases in which testing of a chemical substance would provide scientifically valid and useful information on other chemical substances in the category; and
> (3) the formation of industry consortia to jointly conduct testing to avoid unnecessary duplication of tests, provided that such consortia make all information from such testing available to the Administrator.

The present investigation advances each of these TSCA mandates for chemical substances characterized as surfactants.

A surfactant is a substance that reduces the surface tension of a liquid in which it is dissolved. They are surface-active, amphiphilic compounds that self-assemble to form micelles or aggregates above a critical concentration, referred to as the critical micelle concentration (CMC). These substances are commonly used in industrial processes, occupational settings, and in

consumer products (*e.g.*, household cleaning products, personal care products, *etc.*) as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. The widespread use of surfactants provides opportunities for releases and exposure to human or environmental receptors. The inherent properties of surfactants may induce toxicity if exposures can interfere with biological surfactants or tissues. Certain surfactants are commonly used in a laboratory setting to disrupt cell membranes and denature proteins, which demonstrates the inherent hazards of surfactants. For example, sodium dodecyl sulfate (SDS), a strong anionic surfactant, is used at concentrations up to 10% to disrupt cell membranes and to denature proteins, whereas octylphenoxypolyethoxyethanol , a mild nonionic surfactant, at concentrations up to 1% disrupt cell membranes, while preserving proteins for isolation [ADDIN EN.CITE <EndNote><Cite><Author>Burden</Author><Year>2012</Year><RecNum>14727</RecNum><DisplayText>[1]</DisplayText><record><rec-number>14727</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596017177">14727</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Burden, D.W.</author></authors></contributors><titles><title>Guide to the Disruption of Biological Samples - 2012, Version 1.1.</title><secondary-title>Random Primers</secondary-title></titles><periodical><full-title>Random Primers</full-title></periodical><pages>1-25</pages><number>12</number><dates><year>2012</year></dates><urls></urls></record></Cite></EndNote>].

Hazard concerns for surfactants historically focused on their observed environmental effects and potential toxicity to aquatic organisms based on “down the drain” releases and/or presence in

effluent from wastewater treatment facilities [ADDIN EN.CITE ADDIN EN.CITE.DATA].The EPA has established chemical categories for nonionic, anionic, and cationic (quaternary ammonium) surfactants based on environmental toxicity concerns [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2010</Year><RecNum>14729</RecNum><DisplayText>[3]</DisplayText><record><rec-number>14729</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596017536">14729</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>TSCA New Chemicals Program (NCP) Chemical Categories</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>157, https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf</pages><dates><year>2010</year></dates><urls></urls></record></Cite></EndNote>]. Surfactants may pose a potential hazard to humans, depending on their use and route of exposure, because they can disrupt the normal architecture of the lipid bilayer and reduce the surface tension, thereby solubilizing cell membranes. Mucous membranes are particularly sensitive to the surface-active effects of surfactants, which have been shown to cause irritancy and injury to the eye, based on their ability to “readily penetrate the sandwiched aqueous and lipid barriers of the cornea” [ADDIN EN.CITE <EndNote><Cite><Author>Fox</Author><Year>2008</Year><RecNum>14730</RecNum><

Commented [HT5]: There is a 2010 Category for all 3 Anionic on pg 34, cationic on pg 51 and nonionic, nonionic on pg 94

The categories were established long before 2010; just updated in 2010.

DisplayText>[4]</DisplayText><record><rec-number>14730</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596017801">14730</key></foreign-keys><ref-type name="Book Section">5</ref-type><contributors><authors><author>Fox, D.A.</author><author>Boyes, W.K.</author></authors><secondary-authors><author>Klaassen, C.D.</author></secondary-authors></contributors><titles><title>Toxic Responses of the Ocular and Visual System</title><secondary-title>Casarett & Doull's Toxicology - The Basic Science of Poisons, Seventh Edition</secondary-title></titles><pages>665-697</pages><section>17</section><dates><year>2008</year></dates><pub-location>New York</pub-location><publisher>McGraw-Hill, Medical Publishing Division</publisher><urls></urls></record></Cite></EndNote>].

Depending on the conditions of use, the potential for inhalation exposures to workers and/or consumers warrant consideration in quantitative risk assessments. Surfactants may cause adverse effects on mucous membranes, including the respiratory tract, and interfere with the natural pulmonary surfactants and result in reduction in the oxygen content of arterial blood due to impaired gas exchange in the lung, increases in pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, grossly visible pulmonary edema, and atelectasis [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The chemical category boundary for surfactants that may have the potential to present an inhalation hazard has not been previously defined. The toxicity of surfactants by inhalation exposure can vary over several orders of magnitude, based on their chemical properties. For example, octylphenoxypolyethoxyethanol, (CASRN 9002-93-1) a nonionic surfactant, had a lowest-observed-adverse-effect concentration [LOAEC] of 5.3

mg/m^3) in a 14-day study [ADDIN EN.CITE ADDIN EN.CITE.DATA], while didecyldimethyl ammonium chloride (DDAC; CASRN 7173-51-5), a cationic surfactant and biocide, had a LOAEC of 0.08 mg/m^3 in a 4-week study [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2016</Year><RecNum>14732</RecNum><DisplayText>[10]</DisplayText><record><rec-number>14732</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596018482">14732</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Subchronic Inhalation Toxicity Study of DDAC - Revised</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>25</pages><volume>HQ-OPP-2006-0338-0045</volume><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>] .

The objectives of the present investigation were to: (1) perform a systematic review of the literature with the aim of defining the chemical space for surfactants; (2) identify inhalation toxicity studies on surfactants that may be used to inform inhalation risk assessments; (3) describe scientifically sound new approach methodologies (NAMs) to reduce or replace animal testing; and (4) establish a tiered-testing strategy that uses NAMs to evaluate new chemistries in the Surfactant Category.

MATERIALS AND METHODS

Systematic Literature Review

Two literature searches were performed, an initial search from 1950 through November 2016 and a supplemental search up to April 2018. The details of these searches, including the search strategies, search terms, search results and Population, Exposure, Comparison, and Outcome (PECO) criteria used for reviewing the relevance of the identified studies to this evaluation are provided in the Supporting Information file at "Section 1 Systematic Literature Review". These searchers were conducted with the primary objective of identifying studies that evaluated the toxicity of surfactants in the respiratory tract of humans or laboratory animals, and at the cellular level in *in vitro* and *ex vivo* studies. In addition, these searches were used to identify potential NAMs that could inform a tiered-testing strategy for general surfactants that reduces or replaces the use of vertebrate animals in regulatory testing.

Risk Assessment Approaches under TSCA

Risk Assessment Paradigm

The methods for assessing risks of new chemical substances under TSCA have been developed using science based approaches, scientific peer review, and refinement of the approaches. EPA conducts risk assessments following the four-step process articulated by the U.S. National Research Council (NRC) in 1983 [11] and reaffirmed several times since its initial release [12, 13]. This process includes hazard identification, dose-response analysis, exposure assessment, and risk characterization. Hazard assessment (also called effects assessment in some EPA guidance documents) identifies the adverse health or environmental effects, or hazards, that can

be caused by exposure to a chemical substance. The dose-response analysis assesses the relationship between the exposure or dose of a chemical and the occurrence of health or environmental effects. The exposure assessment characterizes human or environmental exposures, including the magnitude, frequency, and duration, to the extent necessary and practicable within the context of the assessment. Finally, the risk characterization integrates the hazard, dose-response, and exposure components to describe the nature, and when possible, the magnitude of risks to human health and the environment.

The approaches employed for these risk assessment components, including the level of detail and complexity of quantitative aspects, may vary across different risk assessments and typically align with specific legislative and regulatory frameworks. For example, legislative and regulatory frameworks for hazard evaluation of pesticide active ingredients, anti-microbial substances, inerts, *etc*. are described in regulations for pesticides, which include multiple and specific requirements for toxicity data. Under TSCA and its implementing regulations [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2020</Year><RecNum>14738</RecNum><DisplayText>[11]</DisplayText><record><rec-number>14738</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019129">14738</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>40 CFR Part 720 - Premanufacture Notification</title><secondary-title>Code of Federal Regulations</secondary-title></titles><periodical><full-title>Code of Federal Regulations</full-

title></periodical><pages>https://www.law.cornell.edu/cfr/text/40/part-720</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], companies are required to submit a PMN along with available data on: chemical identity, production volume, byproducts, use, environmental release, disposal practices, and human exposure. These submissions are required to include all existing health and environmental data in the possession or control of the submitter, parent company, or affiliates, and a description of any existing data known to or reasonably ascertainable by the submitter. However, TSCA has never included requirements for toxicity testing or generation of hazard data for new chemical substances.

Hazard Assessment

Given the lack of toxicity testing requirements under TSCA, EPA only occasionally receives hazard data for new chemical substances. An analysis of toxicity data submitted to EPA from 2004 through 2012 for new chemical substances found that only about 15% of the PMN submissions included health hazard data; the majority of that information was for acute toxicity (*e.g.*, oral and/or dermal) and irritation (*e.g.*, eye and/or skin) in animals. TSCA provides EPA with the authority to require generation and submission of additional data when the information included with the PMN— coupled with that available to EPA risk assessors from prediction modeling, read-across, internal archives, *etc.* —is insufficient to permit a reasoned evaluation of the health and environmental effects of a new chemical substance. However, prior to making a request for testing using vertebrate animals, EPA must take into consideration reasonably available existing information, including toxicity information; computational toxicology and

bioinformatics; and high-throughput screening methods and the prediction models of those methods (TSCA Section 4(h)(A)(i)-(iii)).

Given the historical lack of hazard data, EPA has, for decades, employed a number of approaches that do not rely on *de novo* toxicity testing. These approaches include computational toxicology (*e.g.*, predictive models and expert systems), analogue[1] read-across wherein available toxicity data for a chemical of similar structure and activity are used to assess the new chemical substance lacking data, and chemical categories (a group of chemicals whose properties are likely to be similar or follow a regular pattern as a result of mechanism, mode of toxic action or structural similarity) [ADDIN EN.CITE <EndNote><Cite><Author>van Leeuwen</Author><Year>2009</Year><RecNum>14739</RecNum><DisplayText>[12]</DisplayText><record><rec-number>14739</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019290">14739</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>van Leeuwen, K.</author><author>Schultz, T. W.</author><author>Henry, T.</author><author>Diderich, B.</author><author>Veith, G. D.</author></authors></contributors><auth-address>TNO Quality of Life, Utrechtseweg 48, The Netherlands.</auth-address><titles><title>Using chemical categories to fill data gaps in hazard assessment</title><secondary-title>SAR QSAR Environ Res</secondary-title><alt-title>SAR and QSAR in environmental research</alt-title></titles><periodical><full-title>SAR

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[1] In the context of this article, an analogue is a chemical substance identified based on its physicochemical and toxicological properties, as one that has undergone evaluation, as stated above, and determined to be an acceptable toxicological analogue for read across to the new chemical substance. An analogue may be directly used in read-across for informing a quantitative risk assessment on a new chemical substance.

QSAR Environ Res</full-title><abbr-1>SAR and QSAR in environmental research</abbr-1></periodical><alt-periodical><full-title>SAR QSAR Environ Res</full-title><abbr-1>SAR and QSAR in environmental research</abbr-1></alt-periodical><pages>207-20</pages><volume>20</volume><number>3-4</number><edition>2009/06/23</edition><keywords><keyword>Hazardous Substances/pharmacology/*toxicity</keyword><keyword>*Quantitative Structure-Activity Relationship</keyword><keyword>Safety Management/*methods</keyword></keywords><dates><year>2009</year></dates><isbn>1026-776x</isbn><accession-num>19544189</accession-num><urls></urls><electronic-resource-num>10.1080/10629360902949179</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]. The integration of these methods with NAMs to advance testing strategies has been recognized by EPA [ADDIN EN.CITE ADDIN EN.CITE.DATA] and is consistent with the vision articulated in the 2007 report by the NRC in "Toxicity Testing in the 21st Century: A Vision and Strategy" [ADDIN EN.CITE <EndNote><Cite><Author>NRC</Author><Year>2007</Year><RecNum>14741</RecNum><DisplayText>[14]</DisplayText><record><rec-number>14741</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019531">14741</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>NRC</author></authors></contributors><titles><title>Toxicity Testing in the 21st Century: A Vision and a Strategy, Washington, D.C. The National

Academies Press</title></titles><pages>216, DOI:
https://doi.org/10.17226/11970</pages><volume>ISBNs: Ebook: 978-0-309-13412-5;
Paperback: 978-0-309-15173-
3</volume><dates><year>2007</year></dates><urls></urls></record></Cite></EndNote>].
EPA defines NAMs "as a broadly descriptive reference to any technology, methodology, approach, or combination thereof that can be used to provide information on chemical hazard and risk assessment that avoids the use of intact animals" [ADDIN EN.CITE
<EndNote><Cite><Author>EPA</Author><Year>2018</Year><RecNum>14844</RecNum><
DisplayText>[15]</DisplayText><record><rec-number>14844</rec-number><foreign-
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trategic Plan to Promote the Development and Implementation of Alternative Test Methods
within the TSCA Program</title><secondary-title>Office of Chemical Safety and Pollution
Prevention & Office of Research and Development, U.S. Environmental Protection Agency,
Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical
Safety and Pollution Prevention & Office of Research and Development, U.S.
Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>39,
https://www.epa.gov/sites/production/files/2018-06/documents/epa_alt_strat_plan_6-20-
18_clean_final.pdf</pages><volume>EPA-740-R1-
8004</volume><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>]

[PAGE]

Dose-Response Analysis

In the absence of test data on new chemical substances, EPA relies on read-across methods using an analogue or a category of analogues to identify hazards and conduct dose-response analysis to identify a point of departure (POD), *i.e.*, a dose or concentration that marks the beginning of a low-dose extrapolation) in the absence of test data on the new chemical substance. EPA's "TSCA New Chemicals Program (NCP) Chemical Categories" [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2010</Year><RecNum>14729</RecNum><DisplayText>[3]</DisplayText><record><rec-number>14729</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596017536">14729</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>TSCA New Chemicals Program (NCP) Chemical Categories</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>157, https://www.epa.gov/sites/production/files/2014-10/documents/ncp_chemical_categories_august_2010_version_0.pdf</pages><dates><year>2010</year></dates><urls></urls></record></Cite></EndNote>] for anionic, nonionic, and cationic surfactants were developed and defined only on environmental toxicity considerations. Toxicity data for analogues are used to identify a POD, such as a no observed adverse effect (concentration) level (NOAE(C)L) or lowest observed adverse effect (concentration) level (LOAE(C)L, for assessing risks of the new chemical substance. This POD can also be the lower

Commented [ST6]: Seems out of order given first sentence, i.e., an analogue and then category

bound on dose (or concentration) for an estimated incidence or a change in response level calculated by a dose-response model such as those available in EPA's benchmark dose software (BMDS), *e.g.*, the BMCL for an observed incidence or change in level of response [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2012</Year><RecNum>14744</RecNum><DisplayText>[16]</DisplayText><record><rec-number>14744</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019975">14744</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Benchmark Dose Technical Guidance</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>99, https://www.epa.gov/sites/production/files/2015-01/documents/benchmark_dose_guidance.pdf</pages><volume>EPA/100/R-12/001</volume><dates><year>2012</year></dates><urls></urls></record></Cite></EndNote>].

EPA has also developed guidance to improve the science underlying the animal-to-human uncertainty factor and provides generalized procedures for deriving dosimetric adjustment factors (DAFs) [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2002</Year><RecNum>14743</RecNum><

DisplayText>[17, 18]</DisplayText><record><rec-number>14743</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019884">14743</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>A Review of the Reference Dose and Reference Concentration Processes</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>192, https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf</pages><volume>EPA/630/P-02/002F</volume><dates><year>2002</year></dates><urls></urls></record></Cite><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-

11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. Application of DAFs to the animal airborne exposure values yields estimates of the concentration that would result in the same concentration to humans, that is, the human equivalent concentration (HEC). Application of a DAF in the calculation of an HEC is considered to address the toxicokinetic aspects of the animal-to-human uncertainty factor (UF) (*i.e.*, to estimate from animal exposure information the human exposure scenario that would result in the same dose to a given target tissue) (EPA, 2002). This operational derivation involves the use of species-specific physiologic and anatomic factors relevant to the form of pollutant (*e.g.*, particle, reactive gas, or volatile organic compound) and categorized with regard to elicitation of response. These factors are all employed in determining the appropriate DAF. For HECs, DAFs are applied to the "duration-adjusted" concentration to which the animals were exposed (*e.g.*, to a weekly average).

For interspecies extrapolation of particle exposures, the Regional Deposited Dose Ratio (RDDR) model developed by EPA can be used to derive a DAF. The RDDR is the ratio of the deposited dose in a respiratory tract region (r) for the laboratory animal species of interest (RDD_A) to that of humans (RDD_H) [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><DisplayText>[18]</DisplayText><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-

type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. EPA's RDDR model allows calculation of RDDR estimates in various regions of the respiratory tract for animals versus humans (*i.e.*, extra-thoracic [ET], tracheobronchial [TB], pulmonary [PU], thoracic [TH], total respiratory tract [RT] and extra-respiratory [ER] regions). The RDDR calculation is based on the characteristics of the aerosol tested in the inhalation study (Median Mass Aerodynamic Diameter or MMAD, Geometric Standard Deviation or GSD, and density), and species-specific parameters for both animals and humans including ventilation rates and regional surface areas of the respiratory tract. The RDDR selected as the DAF is informed by the effects (clinical signs, tissue effects, biochemical changes) observed in the animal toxicity study and the aerosol characteristics in the inhalation study. The DAF is then applied to the duration-adjusted POD to arrive at the HEC of the POD (POD_{HEC}). The RDDR model was used herein to calculate HEC values from the aerosol exposures to animals available for each of the surfactant classes.

After an analogue(s) is identified, the strengths, limitations, and uncertainties associated with the use of the substance(s) to predict the hazards for the new chemical substance are considered when deriving a benchmark margin of exposure (MOE). The benchmark MOE is the result of multiplying all relevant UFs to account for: (1) the variation in susceptibility among the members of the human population (*i.e.*, inter- individual or intraspecies variability); (2) the extrapolation from animal data to humans (*i.e.*, interspecies extrapolation); (3) the extrapolation from data in a study with less- than- lifetime exposure (*i.e.*, extrapolating from sub-chronic to chronic exposure); (4) the extrapolation from a LOAEL to a NOAEL [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2002</Year><RecNum>14743</RecNum><DisplayText>[17, 19]</DisplayText><record><rec-number>14743</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019884">14743</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>A Review of the Reference Dose and Reference Concentration Processes</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>192, https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf</pages><volume>EPA/630/P-02/002F</volume><dates><year>2002</year></dates><urls></urls></record></Cite><Cite><Author>EPA</Author><Year>2014</Year><RecNum>14742</RecNum><record><rec-number>14742</rec-number><foreign-keys><key app="EN" db-

id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019768">14742</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation</title><secondary-title>Office of the Science Advisor, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of the Science Advisor, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>109, https://www.epa.gov/sites/production/files/2015-01/documents/ddef-final.pdf</pages><volume>EPA/R-14/002F</volume><dates><year>2014</year></dates><urls></urls></record></Cite></EndNote>]. EPA prefers using existing information to develop data-derived extrapolation factors (DDEFs) or chemical specific adjustment factors (CSAFs) rather than relying on default values [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2014</Year><RecNum>14742</RecNum><DisplayText>[19]</DisplayText><record><rec-number>14742</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019768">14742</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation</title><secondary-title>Office of the Science Advisor, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C.

20460</secondary-title></titles><periodical><full-title>Office of the Science Advisor, Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>109, https://www.epa.gov/sites/production/files/2015-01/documents/ddef-final.pdf</pages><volume>EPA/R-14/002F</volume><dates><year>2014</year></dates><urls></urls></record></Cite></EndNote>]. This investigation includes several approaches to derive DDEFs to use in assessing new surfactant chemical substances.

Exposure Assessment

Commented [HT7]: Need to consider speaking to Consumer Exposures during peer review

In assessing new chemical substances, generally new chemical substances do not have occupational exposure monitoring data; therefore, EPA typically develops exposure estimates for workers using the Chemical Screening Tool for Exposures and Environmental Releases (ChemSTEER) model. ChemSTEER estimates exposure as daily acute potential dose rates (PDRs) or lifetime average daily doses (LADDs). The PDR represents average exposure over an 8-hour workday, whereas the LADD estimates long-term exposures to the chemical substance, and is averaged over a lifetime exposure of 75 years. The PDR, an initial conservative exposure estimate, is the more appropriate dose-metric for estimating risks to surfactants because surfactants are surface-active at the point of exposure and lung effects occur rapidly following exposure. For chemical substances used in a liquid, mist, or aerosol form, the general default PDR values are 1.875 mg/kg-bw/day for inhalable aerosols or 0.625 mg/kg-bw/day for respirable aerosols as shown in [REF _Ref46930162 \h * MERGEFORMAT] [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2015</Year><RecNum>14745</RecNum><DisplayText>[20]</DisplayText><record><rec-number>14745</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr"

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timestamp="1596021217">14745</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>ChemSTEER User Guide, Chemical Screening Tool for Exposures and Environmental Releases</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>403, https://www.epa.gov/sites/production/files/2015-05/documents/user_guide.pdf</pages><dates><year>2015</year></dates><urls></urls></record></Cite></EndNote>].

Table [SEQ Table * ARABIC]. Default values used for calculating the daily acute potential dose rate (PDR).

Description	Equation	Description	Equation[a]	Defaults	Units
PDR (mg/kg-bw/day)	I/BW	Inhalation PDR (I)	Cm × b × h, where Cm is the mass concentration of chemical in air, b is the volumetric inhalation rate ($0 < b \leq 7.9$), and h is the exposure duration ($0 \leq h \leq 24$)	Cm = 15 mg/m^3 b = 1.25 m^3/hr h = 8 hours/day	mg/day
		Body weight (BW)	BW ($0 \leq BW$)	80 kg-bw	kg-bw

[a] Cm may also be adjusted for the mass concentration of the chemical with a permissible exposure limit (PEL) in air (based on the U.S. Occupational Safety and Health Administration [OSHA] PEL – time-weighted average [TWA]; where: KCk = the mass concentration limit of total particulate in air (mg/m^3) with a default of 15 mg/m^3 for inhalable and 5 mg/m^3 for respirable, Ys= the weight fraction of chemical in particulate ($0 < Ys \leq 1$), Ypel=the weight fraction of chemical or metal in particulate with a known PEL ($0 < Ypel \leq 1$) using the following equation: Cm = KCk × Ys/Ypel

The PDR is calculated using an exposure regimen for a default worker of 8 hours/day and 5 days/week, unless chemical-specific manufacture, processing or use information are provided in the PMN. The exposure conditions in animal studies often do not reflect occupational exposure scenarios; therefore, a duration adjustment and a DAF (*i.e.,* RDDR value) are applied to the POD to derive HECs for exposed human populations according to Agency methods [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><DisplayText>[18]</DisplayText><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. This adjustment would optimally be made using physiologically-based pharmacokinetic modeling [ADDIN EN.CITE

<EndNote><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><DisplayText>[18]</DisplayText><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>], but the data required to conduct such modelling rarely exist for new chemical substances. Therefore, occupational exposures are adjusted using particle deposition models with human ventilation rates during exertion (work) and exposure durations appropriate to the particular occupational setting and chemical use scenario.

Risk Characterization

Risk characterization is the final, integrative step of risk assessment. EPA's Risk Characterization Policy defines risk characterization as the integration of information from the hazard and exposure components of the risk assessment into an overall conclusion about risk that

is complete, informative, and useful for decision-making. The risk characterization conveys the risk assessor's judgment as to the nature and existence of (or lack of) human health or ecological risks [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2000</Year><RecNum>14747</RecNum><DisplayText>[21]</DisplayText><record><rec-number>14747</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021806">14747</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Risk Characterization</title><secondary-title>Office of Science Policy, Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Science Policy, Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>189, https://nepis.epa.gov/Exe/ZyPDF.cgi/40000006.PDF?Dockey=40000006.PDF</pages><volume>EPA 100-B-00-002</volume><dates><year>2000</year></dates><urls></urls></record></Cite></EndNote>]. As described in EPA's Risk Characterization Handbook "Risk characterization at EPA assumes different levels of complexity depending on the nature of the risk assessment being characterized and the level of information contained in each risk characterization varies according to the type of assessment for which the characterization is written and the audience for which the characterization is intended."

Under TSCA section 5, EPA must determine whether a chemical substance presents an unreasonable risk of injury to health or the environment under the conditions of use. EPA generally uses an MOE approach to characterize risks of new chemical substances as a starting point to estimate non-cancer risks for acute and chronic exposures. The MOE is the HEC derived from a POD for a health endpoint (from hazard assessment) divided by the exposure concentration for the scenario of concern (from exposure assessment). The calculated MOE is compared with a benchmark MOE to evaluate whether there is an adequate margin between human exposure estimates and the HEC . When the MOE is less than the benchmark MOE, there is a possibility of human health risks. On the other hand, negligible concerns would be expected if the MOE exceeds the benchmark MOE. The MOE approach is a widely recognized point estimate method and provides a risk profile for different non-cancer health effects and different exposure scenarios.

In summary, in developing a risk assessment for new chemical substances under TSCA section 5, EPA uses empirical data or analogues, to identify a POD(s) and to develop an exposure estimate for use in the evaluation. The hazard assessment in combination with the exposure assessment is used to calculate an MOE, which is compared to the benchmark MOE to identify potential risks. The risk characterization is used to inform the TSCA "unreasonable risk" determination.

RESULTS AND DISCUSSION

Literature Search and Screening Results

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Need to re-write

An initial search of PubMed identified 594 articles that were subjected to title and abstract screening. Of these articles, 551 did not meet the PECO criteria, whereas 43 met the PECO criteria and were selected for full text review. An additional 17 articles that met the PECO criteria awere identified through additional search strategies, screening gray literature, references for other types of chemical substances, *etc*., and were included for full text review. Of the 60 articles evaluated through full text screening, 25 were identified as relevant and carried forward in the present evaluation, whereas the remaining 35 articles were excluded because they lacked relevant information on respiratory tract effects or presented inconclusive epidemiology findings. In the supplemental literature search of PubMed and Embase, 1247 articles (combined) were identified. Following title and abstract screening, 1217 of these articles were excluded because they did not meet the PECO criteria, whereas 25 met the PECO criteria and were selected for full text review. An additional 10 studies that met the PECO criteria were found by additional hand searching), and were selected for full text screening, which resulted in 35 articles that were identified for review; ten articles were deemed irrelevant and excluded. A total of 25 articles were identified in both of the searches, one was excluded because it was in a foreign language and of the remaining 24 articles are summarized in Table 8 in the Supporting Information file at "Section 1 Systematic Literature Review".

~~The initial PubMed search identified 594 articles that were subjected to title and abstract screening. Of these articles, 551 did not meet the PECO criteria, whereas 43 met the PECO criteria and were selected for full text review. An additional 17 articles were included for full text review that met the PECO criteria and were identified through additional search strategies, screening gray literature, references for other types of chemical substances, *etc*., including 9 additional studies found during the supplemental literature search described below. Of the 60~~

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Without the 9 from supplemental was it 8 that were identified from additional strategies?

articles evaluated through full text screening. 25 were identified as relevant and carried forward in the present evaluation, whereas the remaining 35 articles were excluded because they lacked relevant information on respiratory tract effects or presented inconclusive epidemiology findings. In the supplemental literature search, 1247 articles were identified on PubMed and Embase (combined). Following title and abstract screening, 1212 of these articles were excluded because they did not meet the PECO criteria. A total of 35 articles (including 10 studies found by additional hand searching) met the PECO criteria and were selected for full text screening, which resulted in 25 articles that were identified for review; ten articles were deemed irrelevant and excluded. Of the 25 articles identified for review, 9 of the studies were additional studies from the supplemental literature search.

Commented [HT12]: Search 2 = 35
35 – 10 irrelevant = 25

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The information identified in the systematic review was used to determine Category Boundaries and subcategories, to summarize the health effects of surfactants under the section on Hazard Identification, and to identify potential NAMs for use in the Tiered-Testing Strategies.

Category Boundaries

The following structural and functional criteria (hereinafter referred to as the "Surfactant Criteria") are used to distinguish chemical substances, which include polymers and UVCB substances,[2] intended for use as surfactants from other amphiphilic compounds (*e.g.*, ethanol) [ADDIN EN.CITE ADDIN EN.CITE.DATA]:

[2] Chemical Substances of Unknown or Variable Composition, Complex Reaction Products and Biological Materials (UVCB Substance)

1. A substance which has surface-active properties, and which consists of one or more hydrophilic and one or more hydrophobic groups;
2. The substance is capable of reducing the surface tension between air and water to 45 milliNewtons/meter (mN/m) or below at a test condition of 0.5 wt% in water and a temperature of 20°C (*Cf.* Pure water has a surface tension of 72.8 mN/m at 20°C); and
3. The substance self-associates in water to form micellar or vesicular aggregates at a concentration of 0.5 wt% or less.

Commented [HT15]: Condition or Concentration???

Criteria #3 says Concentration

The Surfactants Category is further defined into three general subcategories including nonionic, anionic, and cationic substances. Amphoteric chemical substances that meet the Surfactant Criteria would also be included within these subcategories (*i.e.*, anionic and cationic surfactants), depending on their pH. Lung lining fluids are near neutral pH, with various measurements ranging from 6.6 to 7.1 [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The pKa for each component of an amphoteric surfactant should be evaluated within this pH range and the assessment should be conducted on the predominant components. The non-ionized fraction for acids/bases is calculated as follows:

Commented [HT16]: In Table 3 footnote, called Zwitterionic I vote to change to Amphoteric in Table 3

$$\text{Acids Fraction}_{\text{non-ionized}} = 1 / (1 + 10^{\text{pH-pKa}})$$

$$\text{Bases Fraction}_{\text{non-ionized}} = 1 / (1 + 10^{\text{pKa-pH}})$$

Where the pH represents the physiological pH in the lung lining fluid (*i.e.*, 6.6 to 7.1), and the pKa represents the value for the respective component (*e.g.*, carboxylic acid or amine).

Nonionic surfactants are identified as any neutral chemical substance that meets the Surfactant Criteria. Common nonionic surfactants include alkylphenol chemical substances with one or more ethoxylate (EO) unit as well as linear and branched alcohol chemical substances with one or more EO units. For example, octylphenoxypolyethoxyethanol, a common nonionic octylphenol EO surfactant, and Polysorbate 80 (or Tween 80), another nonionic alkyphenol ethoxylate with increased alkyl chain length and number of EO units, are shown in [REF _Ref47613375 \h * MERGEFORMAT]. The surface tensions of octylphenoxypolyethoxyethanol and Polysorbate 80 range from 30-31 mN/m to 37.96 mN/m, respectively ([REF _Ref47613375 \h * MERGEFORMAT]) [ADDIN EN.CITE <EndNote><Cite><Author>Kothekar</Author><Year>2007</Year><RecNum>14758</RecNum><DisplayText>[28]</DisplayText><record><rec-number>14758</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596025228">14758</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kothekar, S.C.</author><author>Ware, A.M.</author><author>Waghmare, J.T.</author><author>Momin, S.A.</author></authors></contributors><titles><title>Comparative Analysis of the Properties of Tween-20, Tween-60, Tween-80, Arlacel-60, and Arlacel-80</title><secondary-title>Journal of Dispersion Science and Technology</secondary-title></titles><periodical><full-title>Journal of Dispersion Science and Technology</full-title></periodical><pages>477-484, https://www.tandfonline.com/doi/abs/10.1080/01932690601108045</pages><volume>28</volu

me><number>3</number><dates><year>2007</year></dates><urls></urls></record></Cite></EndNote>].

Anionic surfactants are identified as any chemical substance with a net negative charge that meets the Surfactant Criteria (*e.g.*, alkyl sulfonates, alkylbenzene sulfonates, alkylether sulfates, alkyl silicic acids, alkyl phosphates, alkyl carboxylic acids, or combinations of these anionic groups). An example anionic surfactant, SDS, has a reported surface tension of 35 mN/m ([REF _Ref47613375 \h * MERGEFORMAT]).

Cationic surfactants are identified as any chemical substance with a net positive charge that meets the Surfactant Criteria (*e.g.*, alkylammonium chlorides and benzalkonium chlorides). Benzalkonium chloride (BAC: CASRN 8001-54-5) and DDAC are representative members of this subcategory, with surface tensions of 37 mN/m and 25.82 mN/m ([REF _Ref47613375 \h * MERGEFORMAT]), respectively. It is noted that BAC and DDAC also possess biocidal properties.

Typical commercial surfactants (nonionic, anionic, and cationic) are non-volatile liquids or solids. This category framework focuses on exposure *via* aerosol forms (*i.e.*, both airborne droplets and solid particles, including the hygroscopic variety) of these surfactants. While the commercial use of volatile surfactants is unlikely, it should be noted that this framework is not applicable to any substances that qualify as surfactants and are volatile under the conditions of use.

Table [SEQ Table * ARABIC]. Example Chemicals that Meet "Surfactant Criteria" and Nonionic, Anionic and Cationic Subcategorization.

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Nonionic Surfactants					
Chemical Name in Text	**Other Relevant Names**	**Criteria 1**		**Criteria 2**	**Criteria 3**
		Hydrophobic group(s)	**Hydrophilic group(s)**	**Surface Tension**	**Critical Micelle Concentration (CMC)**
formaldehyde, polymer with oxirane and 4-(1,1,3,3-tetramethylbutyl)-phenol Defomaire Alevaire Tyloxapol CASRN: 25301-02-4	CAS Name: formaldehyde, polymer with oxirane and 4-(1,1,3,3-tetramethylbutyl)-phenol	multiple octyl phenol groups	multiple polyoxyethylene (9) units	~37 mN/m at 5 g/L (0.5 wt%) and 25°C* [ADDIN EN.CITE <EndNote><Cite><Author>Schott</Author><Year>1998</Year><RecNum>14754</RecNum><DisplayText>[29]</DisplayText><record><rec-number>14754</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596024000">14754</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Schott,	0.038 g/L or 0.0038 wt% [ADDIN EN.CITE <EndNote><Cite><Author>Schott</Author><Year>1998</Year><RecNum>14754</RecNum><DisplayText>[29]</DisplayText><record><rec-number>14754</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596024000">14754</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sch

				H.</author></authors></contributors><auth-address>School of Pharmacy, Temple University, Philadelphia, Pennsylvania, 19140</auth-address><titles><title>Comparing the Surface Chemical Properties and the Effect of Salts on the Cloud Point of a Conventional Nonionic Surfactant, Octoxynol 9 (Triton X-100), and of Its Oligomer, Tyloxapol (Triton WR-1339)</title><secondary-title>J Colloid Interface Sci</secondary-title><alt-title>Journal of colloid and interface science</alt-title></titles><periodical><full-title>Journal of colloid and interface science</full-title><abbr-1>J Colloid Interface Sci</abbr-	ott, H.</author></authors></contributors><auth-address>School of Pharmacy, Temple University, Philadelphia, Pennsylvania, 19140</auth-address><titles><title>Comparing the Surface Chemical Properties and the Effect of Salts on the Cloud Point of a Conventional Nonionic Surfactant, Octoxynol 9 (Triton X-100), and of Its Oligomer, Tyloxapol (Triton WR-1339)</title><secondary-title>J Colloid Interface Sci</secondary-title><alt-title>Journal of colloid and interface science</alt-title></titles><periodical><full-title>Journal of colloid and interface

				1></periodical><alt-periodical><full-title>Journal of colloid and interface science</full-title><abbr-1>J Colloid Interface Sci</abbr-1></alt-periodical><pages>496-502</pages><volume>205</volume><number>2</number><edition>1998/12/16</edition><dates><year>1998</year><pub-dates><date>Sep 15</date></pub-dates></dates><isbn>0021-9797</isbn><accession-num>9735215</accession-num><urls></urls><electronic-resource-num>10.1006/jcis.1998.5721</electronic-resource-num><remote-database-provider>NLM</remote-database-	science</full-title><abbr-1>J Colloid Interface Sci</abbr-1></alt-periodical><full-title>Journal of colloid and interface science</full-title><abbr-1>J Colloid Interface Sci</abbr-1></alt-periodical><pages>496-502</pages><volume>205</volume><number>2</number><edition>1998/12/16</edition><dates><year>1998</year><pub-dates><date>Sep 15</date></pub-dates></dates><isbn>0021-9797</isbn><accession-num>9735215</accession-num><urls></urls><electronic-resource-num>10.1006/jcis.1998.5721</electronic-resource-

				provider><language>eng</language></record></Cite></EndNote>]	num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]
octylphenoxypolyethoxyethanol CASRN: 9002-93-1	Triton X-100 Octoxynol 9 octylphenol ethoxylate CAS Name: poly(oxy-1,2-ethanediyl), .alpha.-[4-1,1,3,3-tetramethylbutyl)phenyl]-.omega.-hydroxy	octylphenol group	polyoxyethylene (9) unit	~30.5 mN/m at 5 g/L (0.5 wt%) and 25°C* [ADDIN EN.CITE <EndNote><Cite><Author>Schott</Author><Year>1998</Year><RecNum>14754</RecNum><DisplayText>[29]</DisplayText><record><rec-number>14754</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596024000">14754</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Schott, H.</author></authors>	0.17 g/L or 0.017 wt% [ADDIN EN.CITE <EndNote><Cite><Author>Schott</Author><Year>1998</Year><RecNum>14754</RecNum><DisplayText>[29]</DisplayText><record><rec-number>14754</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596024000">14754</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Schott,

				</contributors><auth-address>School of Pharmacy, Temple University, Philadelphia, Pennsylvania, 19140</auth-address><titles><title>Comparing the Surface Chemical Properties and the Effect of Salts on the Cloud Point of a Conventional Nonionic Surfactant, Octoxynol 9 (Triton X-100), and of Its Oligomer, Tyloxapol (Triton WR-1339)</title><secondary-title>J Colloid Interface Sci</secondary-title><alt-title>Journal of colloid and interface science</alt-title></titles><periodical><full-title>Journal of colloid and interface science</full-title><abbr-1>J Colloid Interface Sci</abbr-1></periodical><alt-	H.</author></authors></contributors><auth-address>School of Pharmacy, Temple University, Philadelphia, Pennsylvania, 19140</auth-address><titles><title>Comparing the Surface Chemical Properties and the Effect of Salts on the Cloud Point of a Conventional Nonionic Surfactant, Octoxynol 9 (Triton X-100), and of Its Oligomer, Tyloxapol (Triton WR-1339)</title><secondary-title>J Colloid Interface Sci</secondary-title><alt-title>Journal of colloid and interface science</alt-title></titles><periodical><full-title>Journal of colloid and interface science</full-

				periodical><full-title>Journal of colloid and interface science</full-title><abbr-1>J Colloid Interface Sci</abbr-1></alt-periodical><pages>496-502</pages><volume>205</volume><number>2</number><edition>1998/12/16</edition><dates><year>1998</year><pub-dates><date>Sep 15</date></pub-dates></dates><isbn>0021-9797</isbn><accession-num>9735215</accession-num><urls></urls><electronic-resource-num>10.1006/jcis.1998.5721</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>e	title><abbr-1>J Colloid Interface Sci</abbr-1></periodical><alt-periodical><full-title>Journal of colloid and interface science</full-title><abbr-1>J Colloid Interface Sci</abbr-1></alt-periodical><pages>496-502</pages><volume>205</volume><number>2</number><edition>1998/12/16</edition><dates><year>1998</year><pub-dates><date>Sep 15</date></pub-dates></dates><isbn>0021-9797</isbn><accession-num>9735215</accession-num><urls></urls><electronic-resource-num>10.1006/jcis.1998.5721</electronic-resource-num><remote-

				ng</language></record></Cite></EndNote>]	database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]
polyoxyethylene-10-oleyl ether ($C_{18:1}E_{10}$) CASRN: 9004-98-2	oleyl ethoxylate CAS Name: poly(oxy-1,2-ethanediyl), .alpha.-(9Z)-9-octadecen-1-yl-.omega.-hydroxy	oleyl group	polyoxyethylene (10) unit	35.17 mN/m at 4×10^{-5} M (0.028 wt%) and 25°C* [ADDIN EN.CITE <EndNote><Cite><Author>Liu</Author><Year>2006</Year><RecNum>14761</RecNum><DisplayText>[30]</DisplayText><record><rec-number>14761</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596025582">14761</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Liu,	4×10^{-5} M or 0.028 wt % at 25°C [ADDIN EN.CITE <EndNote><Cite><Author>Liu</Author><Year>2006</Year><RecNum>14761</RecNum><DisplayText>[30]</DisplayText><record><rec-number>14761</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596025582">14761</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Liu,

				F.</author><author>Wang, Z.</author><author>Sun, D.</author><author>Wei, X.</author><author>Zhou, W.</author><author>Li, G.</author><author>Zhang, G.</author></authors></contributors><titles><title>Adsorption Kinetics of Brij 97 at the Air/Solution Interface</title><secondary-title>Journal of Dispersion Science and Technology</secondary-title></titles><periodical><full-title>Journal of Dispersion Science and Technology</full-title></periodical><pages>657-663, https://www.tandfonline.com/doi/abs/10.1080/01932690600660624</pages><volume>27	F.</author><author>Wang, Z.</author><author>Sun, D.</author><author>Wei, X.</author><author>Zhou, W.</author><author>Li, G.</author><author>Zhang, G.</author></authors></contributors><titles><title>Adsorption Kinetics of Brij 97 at the Air/Solution Interface</title><secondary-title>Journal of Dispersion Science and Technology</secondary-title></titles><periodical><full-title>Journal of Dispersion Science and Technology</full-title></periodical><pages>657-663, https://www.tandfonline.com/doi/abs/10.10

				</volume><number>5</number><dates><year>2006</year></dates><urls></urls></record></Cite></EndNote>]	80/019326906006606 24</pages><volume> 27</volume><numbe r>5</number><dates ><year>2006</year> </dates><urls></urls ></record></Cite></ EndNote>]
polyoxyethylene-10-dodecyl ether ($C_{12}E_{10}$) CASRN: 9002-92-0	polyoxyethylene (10) lauryl ether CAS Name: poly(oxy-1,2-ethanediyl),-.alpha.-dodecyl-.omega.-	dodecyl group	polyoxyethylene (10) unit	C12E9: 36 mN/m (concentration not reported) at 23°C* C12E12: 32 mN/m (concentration not reported) at 23°C* [ADDIN EN.CITE <EndNote><Cite><Au thor>Rosen</Author> <Year>1989</Year>< RecNum>14763</Rec Num><DisplayText>[31]</DisplayText><re cord><rec-number>14763</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0 azr5evearxfds0err5sr" timestamp="15960265 43">14763</key></for eign-keys><ref-type name="Edited	12.7×10^{-6} M or 0.0008 wt% at 30°C [ADDIN EN.CITE <EndNote><Cite><A uthor>Sulthana</Aut hor><Year>2000</Y ear><RecNum>1476 2</RecNum><Displa yText>[32]</Display Text><record><rec-number>14762</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre 0azr5evearxfds0err5s r" timestamp="1596025 808">14762</key></ foreign-keys><ref-type name="Journal Article">17</ref-type><contributors>< authors><author>Sult hana,

				Book">28</ref-type><contributors><authors><author>Rosen, M.J.</author></authors></contributors><titles><title>Surfactants and interfacial phenomena</title></titles><pages>431, </pages><dates><year>1989</year></dates><pub-location>New York</pub-location><publisher>John Wiley & Sons, Inc.</publisher><urls></urls></record></Cite></EndNote>]	S.B.</author><author>Rao, P.V.C.</author><author>Bhat, S.G.T.</author><author>Sugihara, N.G.</author><author>Rakshit, A.K.</author></authors></contributors><titles><title>Solution Properties of Nonionic Surfactants and Their Mixtures: Polyoxyethylene (10) Alkyl Ether [CnE10] and MEGA-10</title><secondary-title>Langmuir</secondary-title></titles><periodical><full-title>Langmuir : the ACS journal of surfaces and colloids</full-title><abbr-1>Langmuir</abbr-1></periodical><pages>980-987, https://doi.org/10.1021/la990730o</pages>

					<volume>16</volume><number>3</number><dates><year>2000</year></dates><urls></urls></record></Cite></EndNote>] Also, C12E9 at 1×10^{-6} M at 23°C and C12E12 at 1.4×10^{-6} M at 23°C [ADDIN EN.CITE <EndNote><Cite><Author>Rosen</Author><Year>1989</Year><RecNum>14763</RecNum><DisplayText>[31]</DisplayText><record><rec-number>14763</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596026543">14763</key></foreign-keys><ref-type name="Edited Book">28</ref-type><contributors><authors><author>Rosen,

					M.J.</author></authors></contributors><titles><title>Surfactants and interfacial phenomena</title></titles><pages>431, </pages><dates><year>1989</year></dates><pub-location>New York</pub-location><publisher>John Wiley & Sons, Inc.</publisher><urls></urls></record></Cite></EndNote>]
Polysorbate 20 (Tween 20) CASRN: 9005-64-5	polyoxyethylene (20) sorbitan monolaurate CAS Name: sorbitan, monododecanoate, poly(oxy-1,2-ethanediyl) derivs.	dodecanoyl group	sorbitan polyoxyethylene (20) unit	38 mN/m at 8.04×10^{-5} M (0.001 wt%) and 21°C* [ADDIN EN.CITE <EndNote><Cite><Author>Kim</Author><Year>2001</Year><RecNum>14756</RecNum><DisplayText>[33]</DisplayText><record><rec-number>14756</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0	8.04×10^{-5} M or 0.001 wt% at 21°C [ADDIN EN.CITE <EndNote><Cite><Author>Kim</Author><Year>2001</Year><RecNum>14756</RecNum><DisplayText>[33]</DisplayText><record><rec-number>14756</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5s

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				r>31</number><dates><year>2001</year></dates><urls></urls></record></Cite></EndNote>]	>187-188</volume><number>31</number><dates><year>2001</year></dates><urls></urls></record></Cite></EndNote>]
Polysorbate 80 (Tween 80) CASRN: 9005-65-6	polyoxyethylene (20) sorbitan monooleate CAS Name: sorbitan, mono-(9Z)-9-octadecenoate, poly(oxy-1,2-ethanediyl) derivs.	octadecenoyl group	sorbitan polyoxyethylene (20) unit	37.96 mN/m at 5 g/L (0.5 wt%) and 30°C [ADDIN EN.CITE <EndNote><Cite><Author>Kothekar</Author><Year>2007</Year><RecNum>14758</RecNum><DisplayText>[28]</DisplayText><record><rec-number>14758</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596025228">14758</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kothekar, S.C.</author><author>Ware,	1.5×10^{-5} M or 0.002 wt% at 25°C [ADDIN EN.CITE <EndNote><Cite><Author>Mahmood</Author><Year>2013</Year><RecNum>14757</RecNum><DisplayText>[34]</DisplayText><record><rec-number>14757</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596024783">14757</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Mahmood, M.E.</author><autho

				A.M.</author><author>Waghmare, J.T.</author><author>Momin, S.A.</author></authors></contributors><titles><title>Comparative Analysis of the Properties of Tween-20, Tween-60, Tween-80, Arlacel-60, and Arlacel-80</title><secondary-title>Journal of Dispersion Science and Technology</secondary-title></titles><periodical><full-title>Journal of Dispersion Science and Technology</full-title></periodical><pages>477-484, https://www.tandfonline.com/doi/abs/10.1080/01932690601108045</pages><volume>28</volume><number>3</number><dates><year>2007</year></dates><urls></urls></reco	r>Al-Koofee, D.A.F.</author></authors></contributors><titles><title>Effect of Temperature Changes on Critical Micelle Concentration for Tween Series Surfactants</title><secondary-title>Global Journal of Science Frontier Research Chemistry</secondary-title></titles><periodical><full-title>Global Journal of Science Frontier Research Chemistry</full-title></periodical><pages>5, https://journalofscience.org/index.php/GJSFR/article/view/816/681</pages><volume>13(B)</volume><number>4</number><dates><year>2013</year></dates><urls></urls></record></Cite></EndNote>]

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				rd></Cite></EndNote >]	
Poloxamer 188 CASRN: 691397-13-4	CAS Name: oxirane, 2-methyl-, polymer with oxirane, triblock	polyoxypropylene (27) unit	two polyoxyethylene (80) units	~42-44 mN/m at ~0.5 wt% and 36°C [ADDIN EN.CITE ADDIN EN.CITE.DATA]	4.8×10^{-4} M or 0.4 wt% at 37°C [ADDIN EN.CITE ADDIN EN.CITE.DATA]
N,N-dimethyl-dodecylamine-N-oxide ($C_{12}AO$)*** CASRN: 1643-20-5	lauryl dimethylamine oxide CAS Name:1-dodecanamine, N,N-dimethyl-, N-oxide	dodecyl group	amine oxide unit	34.1 mN/m at 1 g/L (0.1 wt%) and 20°C [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14772</RecNum><DisplayText>[37]</DisplayText><record><rec-number>14772</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596028055">14772</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></aut	1.7×10^{-3} M or 0.039 wt% [ADDIN EN.CITE <EndNote><Cite><Author>Hoffmann</Author><Year>1990</Year><RecNum>14764</RecNum><DisplayText>[38]</DisplayText><record><rec-number>14764</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596026736">14764</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hoffmann,

				hors></contributors><titles><title>Dodecyldimethylamine oxide, CASRN: 1643-20-5, EC number: 216-700-6, Surface Tension</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/registration-dossier/-/registered-dossier/10062/4/11</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]	H.</author></authors></contributors><titles><title>Correlation between surface and interfacial tensions with micellar structures and properties of surfactant solutions</title><secondary-title>Progress in Colloid & Polymer Science</secondary-title></titles><periodical><full-title>Progress in Colloid & Polymer Science</full-title></periodical><pages>16-28, https://link.springer.com/chapter/10.1007%2FBFb0116238</pages><volume>18</volume><dates><year>1990</year></dates><urls></urls></record></Cite></EndNote>] 1×10^{-5} M to 5.5×10^{-5} M or 0.0002 to 0.001

					wt% at 25°C [ADDIN EN.CITE <EndNote><Cite><Author>Mukerjee</Author><Year>1971</Year><RecNum>14765</RecNum><DisplayText>[39]</DisplayText><record><rec-number>14765</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596026897">14765</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Mukerjee, P.</author><author>Mysels, K.J.</author></authors></contributors><titles><title>Critical micelle concentrations of aqueous surfactant systems</title><seco

					ndary-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</secondary-title></titles><periodical><full-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</full-title></periodical><pages>242, https://nvlpubs.nist.gov/nistpubs/Legacy/NSRDS/nbsnsrds36.pdf</pages><dates><year>1971</year></dates><urls></urls></record></Cite></EndNote>]
Anionic Surfactants					
Chemical	**Other Relevant Names**	**Criteria 1**		**Criteria 2**	**Criteria 3**

Name in Text		Hydrophobic group(s)	Hydrophilic group(s)	Surface Tension	Critical Micelle Concentration (CMC)
sodium dodecyl sulfate (SDS) CASRN: 151-21-3	CAS Name: sulfuric acid monododecyl ester sodium salt (1:1)	dodecyl group	sulfate group	35 mN/m at 0.29 wt% and 20°C [ADDIN EN.CITE <EndNote><Cite><Author>Hernainz</Author><Year>2002</Year><RecNum>14768</RecNum><DisplayText>[40]</DisplayText><record><rec-number>14768</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596027363">14768</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hernainz, F.</author><author>Caro, A.</author></authors></contributors><titles><title>Variation of surface tension in	8.25×10^{-3} M or 0.24 wt% at 20°C [ADDIN EN.CITE <EndNote><Cite><Author>Mukerjee</Author><Year>1971</Year><RecNum>14765</RecNum><DisplayText>[39]</DisplayText><record><rec-number>14765</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596026897">14765</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Mukerjee, P.</author><author>Mysels, K.J.</author></authors></contributors><titles><title>Critical

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				aqueous solutions of sodium dodecyl sulfate in the flotation batch</title><secondary-title>Colloids and Surfaces A: Physicochemical and Engineering Aspects</secondary-title></titles><periodical><full-title>Colloids and Surfaces A: Physicochemical and Engineering Aspects</full-title></periodical><pages>19-24, https://www.sciencedirect.com/science/article/abs/pii/S0927775701005751</pages><volume>196</volume><number>1</number><dates><year>2002</year></dates><urls></urls></record></Cite></EndNote>]	micelle concentrations of aqueous surfactant systems</title><secondary-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</secondary-title></titles><periodical><full-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</full-title></periodical><pages>242, https://nvlpubs.nist.gov/nistpubs/Legacy/NSRDS/nbsnsrds36.pdf</pages><dates><year>1971</year></dates><urls></urls></record></Cite></EndNote>]

oleoyl sarcosine CASRN: 110-25-8	CAS Name: glycine, N-methyl-N-((9Z)-1-oxo-9-octadecen-1-y	oleyl group	carboxylic acid anion	31.91 mN/m at 0.1 wt% and 19.9°C** [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14767</RecNum><DisplayText>[41]</DisplayText><record><rec-number>14767</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596027202">14767</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sodium N-methyl-N-(1-oxo-9-octadecenyl)aminoacetate, CASRN 3624-77-9, EC number: 222-829-9, Surface Tension</title><secon	2.6×10^{-3} wt% and ~25°C ** (temperature not reported, assumed to be room temperature) [ADDIN EN.CITE <EndNote><Cite><Author>ChattemChemicals</Author><Year>2020</Year><RecNum>14769</RecNum><DisplayText>[42]</DisplayText><record><rec-number>14769</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596027596">14769</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>ChattemChemicals</author></authors></contributors><titles><title>Oleoyl Sarcosine, CASRN 110-25-

				dary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://www.echa.europa.eu/fi/web/guest/registration-dossier/-/registered-dossier/5350/4/11</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]	8</title><secondary-title>Product Information</secondary-title></titles><periodical><full-title>Product Information</full-title></periodical><pages>https://www.chattemchemicals.com/</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]
sodium lauroyl sarcosinate CASRN: 137-16-6	CAS Name: glycine, N-methyl-N-(1-oxododecyl)-, sodium salt (1:1)	lauryl group	carboxylic acid anion	40.5 mN/m at 2 wt% and 20°C [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14770</RecNum><DisplayText>[43]</DisplayText><record><rec-number>14770</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0	8.0×10^{-2} wt% and ~25°C (temperature not reported, assumed to be room temperature) [ADDIN EN.CITE <EndNote><Cite><Author>ChattemChemicals</Author><Year>2020</Year><RecNum>14769</RecNum><DisplayText>[42]</DisplayText><record><rec-number>14769</rec-

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				rls></urls></record></Cite></EndNote>]	ord></Cite></EndNote>]
dioctyl sulfosuccinate sodium salt (DOSS) CASRN: 577-11-7	dioctyl sodium sulfosuccinate CAS Name: Butanedioic acid, 2-sulfo-, 1,4-bis(2-ethylhexyl) ester, sodium salt	two 2-ethyl hexyl groups	sulfosuccinate group	<28 mN/m at 0.5 vol% and 25°C* [ADDIN EN.CITE <EndNote><Cite><Author>Williams</Author><Year>1957</Year><RecNum>14755</RecNum><DisplayText>[44]</DisplayText><record><rec-number>14755</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596024180">14755</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Williams, E.F.</author><author>Woodberry, N.T.</author><author>Dixon, J.K.</author></authors></contributors><titles><title>Purification	6.8×10^{-4} M or 0.03 wt% at 25°C [ADDIN EN.CITE <EndNote><Cite><Author>Mukerjee</Author><Year>1971</Year><RecNum>14765</RecNum><DisplayText>[39]</DisplayText><record><rec-number>14765</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596026897">14765</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Mukerjee, P.</author><author>Mysels, K.J.</author></authors></contributors><titles><title>Critical micelle

				and surface tension properties of alkyl sodium sulfosuccinates</title><secondary-title>Journal of Colloid Science</secondary-title></titles><periodical><full-title>Journal of Colloid Science</full-title></periodical><pages>452-459</pages><volume>12</volume><number>5</number><dates><year>1957</year></dates><urls></urls></record></Cite></EndNote>]	concentrations of aqueous surfactant systems</title><secondary-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</secondary-title></titles><periodical><full-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC 20234</full-title></periodical><pages>242, https://nvlpubs.nist.gov/nistpubs/Legacy/NSRDS/nbsnsrds36.pdf</pages><dates><year>1971</year></dates><urls></urls></record></Cite></EndNote>]

Cationic Surfactants					
Chemical Name in Text	**Other Relevant Names**	**Criteria 1**		**Criteria 2**	**Criteria 3**
		Hydrophobic group(s)	**Hydrophilic group(s)**	**Surface Tension**	**Critical Micelle Concentration (CMC)**
benzalkonium chloride (BAC) CASRN: 8001-54-5	CAS Name: quaternary ammonium compounds, alkylbenzyldimethyl, chlorides	alkyl chains are C12, C14, C16 and C18 and benzyl group	quaternary nitrogen	37 mN/m at concentrations greater than about 4×10^{-4}M and 25°C* [ADDIN EN.CITE <EndNote><Cite><Author>Nandni</Author><Year>2013</Year><RecNum>14766</RecNum><DisplayText>[45]</DisplayText><record><rec-number>14766</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596027033">14766</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Nandni,	C12: reported values range from 2.3 - 8.5×10^{-3} M or 0.078 - 0.29 wt% at 25°C C14: 3.7×10^{-4} M or 0.014 wt% and ~25°C (temperature not stated; assumed to be room temperature) C16: 4.2×10^{-5} M or 0.0016 wt% at 23°C C18: reported values range from 7.1 - 8.5×10^{-6} M or 0.0003 - 0.00036 wt% at 23°C [ADDIN EN.CITE <EndNote><Cite><Author>Mukerjee</Author><Year>1971</Year><RecNum>14765</RecNum><DisplayText>[39]</Display

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				D.</author><author>Mahajan, R.K.</author></authors></contributors><titles><title>Micellar and Interfacial Behavior of Cationic Benzalkonium Chloride and Nonionic Polyoxyethylene Alkyl Ether Based Mixed Surfactant Systems</title><secondary-title>Journal of Surfactants and Detergents</secondary-title></titles><periodical><full-title>Journal of Surfactants and Detergents</full-title></periodical><pages>587-599, https://doi.org/10.1007/s11743-012-1427-z</pages><volume>16</volume><number>4</number><dates><year>2013</year></dates><urls></urls></record></Cite></EndNote>]	Text><record><rec-number>14765</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596026897">14765</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Mukerjee, P.</author><author>Mysels, K.J.</author></authors></contributors><titles><title>Critical micelle concentrations of aqueous surfactant systems</title><secondary-title>Prepared under contract for the Office of Standard Reference Data, National Bureau of Standards of NSRDS-NBS 36, Washington, DC

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didecyldimethyl ammonium chloride (DDAC) CASRN: 7173-51-5	CAS Name: 1-decanaminium, N-decyl-N,N-dimethyl-, chloride (1:1)	decyl groups	quaternary nitrogen	25.82 mN/m at 1 g/L (0.1 wt%) and 20°C [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14771</RecNum><DisplayText>[46]</DisplayText><record><rec-number>14771</rec-number><foreign-	0.39 g/L or 0.039 wt% at 25°C [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14771</RecNum><DisplayText>[46]</DisplayText><record><rec-number>14771</rec-number><foreign-

				keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596027946">14771</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Didecyldimethylammonium chloride, CASRN: 7173-51-5, EC number: 230-525-2, Surface Tension</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/registration-dossier/-/registered-	keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596027946">14771</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Didecyldimethylammonium chloride, CASRN: 7173-51-5, EC number: 230-525-2, Surface Tension</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/registration-

				dossier/5864/4/11</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]	dossier/-/registered-dossier/5864/4/11</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]

*Not all of the surface tension measurement references identified are run at exactly 20°C, but they are sufficiently close (within 5°C) so as not to affect the measurement. In addition, several measurements were run at 0.1% instead of the recommended 0.5%. Increasing the concentration to 0.5% is likely to lower the surface tension.

**Carboxylic acid compounds, such as oleoyl sarcosine, have a carboxyl group pKa value of ~5, thus at physiological pH values maintained near 7 in the lung, the carboxyl group will be 99% in the anionic form according the Henderson-Hasselbalch equation. Since sodium is the major cation in mammalian body fluids (~145 mM), the use of the sodium oleoyl sarcosine surface tension value is appropriate for its characterization.

***Zwitterionic: At pH 7, 90% expected to be nonionic; only small amount cationic.

Commented [HT18]: This is called Amphoteric in the general category explanation above

Hazard Identification

There is concern for dysfunction of mucus, epithelial lining fluid, and natural surfactant lining in the various regions of the respiratory tract from inhalation of surfactants. There is also evidence that some surfactants or similar structures may also interfere with the cell membrane of the epithelium in these same regions [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The capacity of exogenous surfactants to interfere with pulmonary surfactant and impair pulmonary function has been demonstrated in both human volunteers and in laboratory animals [51, 5-7]. The respiratory tract responses to inhaled surfactant aerosol is thought to be in proportion to the exposure concentration and duration, but available data on acute and repeated-dose effect levels are limited within each subcategory, which limits establishing a correlation between chemical properties and toxicity due to exposure methods (*e.g.,* generated aerosol droplet size).

Nonionic Surfactants

In vivo studies

Several studies were identified for the nonionic siliconized superinone respiratory detergent, formaldehyde, polymer with oxirane and 4-(1,1,3,3-tetramethylbutyl)phenol polymer (CASRN 25301-02-4; commonly known as Defomarie, Alevaire, and Tyloxapol). Healthy human volunteers demonstrated significantly decreased respiratory compliance following acute inhalation of Defomaire [ADDIN EN.CITE <EndNote><Cite><Author>Obenour</Author><Year>1963</Year><RecNum>13656</RecNum><DisplayText>[49]</DisplayText><record><rec-number>13656</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr"

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SALTZMAN, H A
SIEKER, H O
GREEN, J L
1963/11/01</edition><keywords><keyword>Aerosols</keyword><keyword>Alcohols</keyword><keyword>Ethanol</keyword><keyword>Heart Failure</keyword><keyword>Humans</keyword><keyword>Infusions, Parenteral</keyword><keyword>Injections, Intravenous</keyword><keyword>Lung</keyword><keyword>Lung Compliance</keyword><keyword>Pulmonary Edema</keyword><keyword>Respiratory Function Tests</keyword><keyword>Silicones</keyword><keyword>Sodium Chloride</keyword><keyword>Surface-Active Agents</keyword></keywords><dates><year>1963</year><pub-dates><date>Nov</date></pub-dates></dates><isbn>0009-7322 (Print)
0009-7322 (Linking)</isbn><accession-num>14079193</accession-num><call-num>0 (Aerosols)
0 (Alcohols)
0 (Silicones)
0 (Surface-Active Agents)
3K9958V90M (Ethanol)
451W47IQ8X (Sodium Chloride)</call-num><urls></urls><remote-database-

provider>NLM</remote-database-provider><language>Eng</language></record></Cite></EndNote>]. An increased minimum surface tension due to detergent was shown to be dose-dependent, using pulmonary surfactant extracted from dogs with the nonionic surfactant tyloxapol (Alevaire) *in vitro* [ADDIN EN.CITE ADDIN EN.CITE.DATA]. However, *in vivo* exposure of dogs to Alevaire (8 hour aerosol exposure; vehicle and concentration not reported) produced little effect (only 1/10 dogs exposed to Alevaire showed increased minimum surface tension). The results did not support the dose-dependence of the effect and indicated that small amounts of detergent in the lungs may not detectably alter the surface tension-surface area relationship and that alteration of surface tension is unlikely to occur during reasonable use [ADDIN EN.CITE ADDIN EN.CITE.DATA].

Inhalation studies using dogs and/or sheep exposed to nonionic surfactant, tyloxapol, resulted in reduced oxygen content of arterial blood due to impaired gas exchange in the lung, increased pulmonary extravascular water volume and wet-to-dry weight ratio of the lungs, and grossly visible pulmonary edema and atelectasis (*i.e.*, collapsed alveoli) [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In the study by Modell *et al.* (1969) [ADDIN EN.CITE ADDIN EN.CITE.DATA], no gross pathology differences were seen in detergent-exposed versus control lungs of dogs, although some portions of both control and exposed lungs were heavy and discolored reddish-purple, which may have been caused by fluid accumulation from the liquid aerosol exposures and/or the use of hypotonic saline in the study (0.45% NaCl) since these effects were not observed in lungs treated with a less dense aerosol. Normal appearances were observed in the remaining areas of the lungs.

Commented [HT19]: ?? why calling this 'alcohol' here...the Surfactant Criteria are supposed to **distinguish** a surfactant from other amphiphilic chemicals like alcohols

In rodents, irritation and inflammatory effects in the entire respiratory tract have been observed with varying degrees of severity. Acute inhalation exposure *via* nose-only administration for 4 hours in Wistar Han rats to a concentration of 5.1 mg/L (5,100 mg/m^3) to ~~Polysorbate 20~~Sorbitan monolaurate, ethoxylated (~~Tween 20~~ CASRN 9005-64-5), a chemical not irritating to the skin or eyes [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14776</RecNum><DisplayText>[50]</DisplayText><record><rec-number>14776</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596030693">14776</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sorbitan monolaurate, ethoxylated, 1 - 6.5 moles ethoxylated, CASRN: 9005-64-5, EC number: 500-018-3, Skin irritation/corrosion</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/13525/7/4/2</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], with an MMAD of 2.2 µm and a GSD of 2, did not result in an increase in mortalities, clinical signs, or abnormalities in the gross pathology [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14777</RecNum><DisplayText>[51]</DisplayText><record><rec-number>14777</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596030813">14777</key></foreign-keys><ref-type name="Journal

Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sorbitan monolaurate, ethoxylated 1 - 6.5 moles ethoxylated, CASRN: 9005-64-5, EC number: 500-018-3, Acute Toxicity: Inhalation</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/13525/7/3/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. A respiratory irritation study was performed on a mixture containing octylphenoxypolyethoxyethanol (CASRN 9002-93-1) [ADDIN EN.CITE ADDIN EN.CITE.DATA], which can be severely irritating to the skin and eyes, in male Webster mice exposed for 3 hours to concentrations of 12, 22, 51, 118, and 134 mg/m^3 with 30-60 minutes recovery time (MMAD and GSD not provided). Signs of pulmonary irritation were observed in animals at the two highest concentrations as indicated by a decrease in respiratory frequency; this response was preceded by an increase in respiratory frequency at the highest three concentrations without an increase in gross lung abnormalities, pulmonary edema, or lung weight [ADDIN EN.CITE <EndNote><Cite><Author>Alarie</Author><Year>1992</Year><RecNum>14778</RecNum><DisplayText>[52]</DisplayText><record><rec-number>14778</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596035219">14778</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Alarie, Y.</author><author>Stock, M.F.</author></authors></contributors><titles><title>Respiratory Irritancy on a Mixture containing Polyethylene Glycol Mono(Octyl)Phenyl Eether CAS #9035-19-5</title><secondary-

title>ChemView - U.S. Environmental Protection Agency</secondary-title></titles><periodical><full-title>ChemView - U.S. Environmental Protection Agency</full-title></periodical><pages>37, https://chemview.epa.gov/chemview/proxy?filename=09022526800b76c9_86960000465_09-26-2011_8D_PHCS_Original%20-%2086960000465.pdf</pages><dates><year>1992</year></dates><urls></urls></record></Cite></EndNote>]. An acute inhalation exposure study in Syrian hamsters exposed to 3.0 mg/L of octylphenoxypolyethoxyethanol with varying exposure durations showed that lung deposition directly corresponded to mortality with an LD_{50} of 1300-2100 µg with an MMAD of 1.47 µm and a GSD of 1.84 [ADDIN EN.CITE <EndNote><Cite><Author>Damon</Author><Year>1982</Year><RecNum>13323</RecNum><DisplayText>[53]</DisplayText><record><rec-number>13323</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1479320592">13323</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Damon, E. G.</author><author>Halliwell, W. H.</author><author>Henderson, T. R.</author><author>Mokler, B. V.</author><author>Jones, R. K.</author></authors></contributors><titles><title>Acute toxicity of polyethylene glycol p-isooctylphenol ether in syrian hamsters exposed by inhalation or bronchopulmonary lavage</title><secondary-title>Toxicology and applied pharmacology</secondary-title><alt-title>Toxicol Appl Pharmacol</alt-title></titles><periodical><full-title>Toxicology and Applied Pharmacology</full-title><abbr-1>Toxicol. Appl. Pharmacol.</abbr-1></periodical><pages>53-61</pages><volume>63</volume><number>1</number><edition>Damon, E

G
Halliwell, W H
Henderson, T R
Mokler, B V
Jones, R K
1982/03/30</edition><keywords><keyword>Animals</keyword><keyword>Cricetinae</keyword><keyword>Detergents/ toxicity</keyword><keyword>Dose-Response Relationship, Drug</keyword><keyword>Female</keyword><keyword>Lethal Dose 50</keyword><keyword>Lung/ drug effects/pathology</keyword><keyword>Male</keyword><keyword>Mesocricetus</keyword><keyword>Octoxynol</keyword><keyword>Polyethylene Glycols/administration & dosage/ toxicity</keyword><keyword>Surface-Active Agents/ toxicity</keyword><keyword>Therapeutic Irrigation</keyword></keywords><dates><year>1982</year><pub-dates><date>Mar 30</date></pub-dates></dates><isbn>0041-008X (Print)
0041-008X (Linking)</isbn><accession-num>7071873</accession-num><call-num>0 (Detergents)
0 (Surface-Active Agents)
30IQX730WE (Polyethylene Glycols)
9002-93-1 (Octoxynol)</call-num><urls></urls><remote-database-provider>NLM</remote-database-provider><language>Eng</language></record></Cite></EndNote>]. The authors concluded that the deaths in these animals were likely the result of severe laryngeal edema and ulcerative laryngitis while the lower airways in these animals were relatively free of serious pathologies. The authors hypothesized that these observed effects were due to large tracheobronchial deposition following the aerosol exposure and the mucociliary clearance of the chemical resulted in a large concentration on the laryngeal mucosa, though laryngeal deposition is typically a function of aerodynamics. In the only 2-week whole-body inhalation study for nonionic surfactants, male and female Sprague-Dawley rats were exposed to 5.3 and 10.3 mg/m^3 (5/sex/dose; MMAD 1.8 µm, GSD 1.8) octylphenoxypolyethoxyethanol for 6 hours/day, 5

days/week [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Slight to minimal subacute inflammation of the alveolar walls and hyperplasia of the alveolar/bronchiolar epithelium was reported, in addition to an increase in slight discoloration of the lungs, increased lung weight, and mucoid nasal discharge; a LOAEC of 5.3 mg/m^3 was identified.

Mechanistic studies

In vitro studies of surfactant on cell membranes have provided evidence of possible modes of action (MOAs). Warisnoicharoen *et al.* (2003) [ADDIN EN.CITE ADDIN EN.CITE.DATA] evaluated the cytotoxicity of the nonionic surfactants polyoxyethylene-10-oleyl ether ($C_{18:1}E_{10}$; CASRN 9004-98-2), polyoxyethylene-10-dodecyl ether ($C_{12}E_{10}$; CASRN 9002-92-0), and N,N-dimethyl-dodecylamine-N-oxide ($C_{12}AO$; CASRN 1643-20-5) on submerged cultured human bronchial epithelium cells (16-HBE14o-) *in vitro*, using the MTT cell viability assay by exposing the cells to 0.1mL of the serially diluted microemulsion for 30 minutes followed by a 60 minute incubation with a MTT solution (particle size not reported). All surfactants tested were cytotoxic at concentrations near or below their critical aggregation (micellular) concentrations (as determined by surface tension measurements), suggesting that toxicity was due to the disruption caused by the partitioning of monomeric surfactant into the cell membrane.

Lindenberg *et al.* (2019) [ADDIN EN.CITE <EndNote><Cite><Author>Lindenberg</Author><Year>2019</Year><RecNum>14779</RecNum><DisplayText>[55]</DisplayText><record><rec-number>14779</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr"

timestamp="1596035601">14779</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Lindenberg, F.</author><author>Sichel, F.</author><author>Lechevrel, M.</author><author>Respaud, R.</author><author>Saint-Lorant, G.</author></authors></contributors><titles><title>Evaluation of Lung Cell Toxicity of Surfactants for Inhalation Route</title><secondary-title>Journal of Toxicology and risk assessment</secondary-title></titles><periodical><full-title>Journal of Toxicology and risk assessment</full-title></periodical><pages>https://doi.org/10.23937/2572-4061.1510022</pages><volume>5</volume><number>1</number><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>] evaluated the cytotoxic activity of the three nonionic polymeric surfactants Polysorbate 20, Polysorbate 80 (Tween 80; CASRN 9005-65-6), and Poloxamer 188 (CASRN 691397-13-4), which are commonly used in formulations of nebulized pharmaceuticals to prevent protein agglomeration, in a BEAS-2B human bronchial epithelial cell model using an innovative air-liquid interface (ALI) method of exposure to surfactants with a nasal spray system (MMAD and GSD not provided). In this study, the ALI results were compared to the classical submerged cell culture or liquid/liquid (L/L) model. The study measured the release of Lactate Dehydrogenase (LDH), an intercellular enzyme present in the cytoplasm, indicative of the loss of membrane integrity. Cytotoxicity of Polysorbate 20 was observed at concentrations of 1-2% (v/v) when using the more biologically relevant ALI method; however, a significant increase in LDH was only observed at 4% for Polysorbate 80 and not significantly increased at concentrations of up to 10% for Poloxamer 188. These results suggest that Polysorbate 20 and to a lesser extent, Polysorbate 80 induce damage to the cell membrane integrity while the linear Poloxamer 188 did not demonstrate any *in vitro* cytotoxicity.

The available *in vitro* and *in vivo* data indicate inconsistency in respiratory toxicity among nonionic surfactants; however, the degree to which the variation is due to experimental design or bioactivity of the surfactant is not discernible from these data. The small dataset presented in this section preclude establishing correlations between respiratory effects and chemical properties, such as surface tension or CMC. Similarly, the examination of the relationship between chemical properties of nonionic surfactants and eye irritation has not established that hydrophilic-lipophilic balance, pH, alkyl chain length, or poly [oxyethylene] chain lengths can be used to predict eye irritation potential across the nonionic surfactant subcategory [ADDIN EN.CITE <EndNote><Cite><Author>Heinze</Author><Year>1999</Year><RecNum>14780</RecNum><DisplayText>[56]</DisplayText><record><rec-number>14780</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596035990">14780</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Heinze, J.E.</author><author>Casterton, P.L.</author><author>Atrash, J.</author></authors></contributors><titles><title>Relative Eye Irritation Potential of Nonionic Surfactants: Correlation to Dynamic Surface Tension</title><secondary-title>Journal of toxicology: cutaneous and ocular toxicology</secondary-title></titles><periodical><full-title>Journal of toxicology: cutaneous and ocular toxicology</full-title></periodical><pages>359-374, https://doi.org/10.3109/15569529909065552</pages><volume>18</volume><dates><year>1999</year></dates><urls></urls></record></Cite></EndNote>]. However, significant correlations of eye irritation and the maximum reduction in surface tension were observed at the CMC or

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Changed to inconsistency

higher surfactant concentration when surface tension was measured under dynamic conditions (0.24, 1, and 4 bubbles/second). Whether this chemical property similarly predicts potency of nonionic surfactants for respiratory effects requires additional data and analysis outside of the scope of this summary.

Anionic Surfactants

In vivo studies

Two acute inhalation toxicity studies were identified for anionic surfactants, which demonstrated high toxicity *via* the inhalation route. Oleoyl sarcosine (CASRN 110-25-8), irritating to the skin and damaging to the eye [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14781</RecNum><DisplayText>[57]</DisplayText><record><rec-number>14781</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596036160">14781</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>N-methyl-N-[C18-(unsaturated)alkanoyl]glycine, CASRN: NA, EC number: 701-177-3, Skin irritation/corrosion</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/21429/7/4/2/?documentUUID=fbaef057-ecc7-4763-aa56-1fa2c88c606c</pages><dates><year>2020</year></dates><urls></urls></record></Cite></End

Note>], was evaluated in a 4-hour nose-only inhalation study in male and female Sprague-Dawley rats at concentrations of 0.3, 0.6, 2.2, and 3.7 mg/L (300, 600, 2,200, 3,700 mg/m^3). The MMAD and GSD were not reported. An LC_{50} of 1.37 mg/L was identified with edema of the lung at 0.6 mg/L and audible gasping at 0.3 mg/L. For sodium lauroyl sarcosinate (CASRN 137-16-6), irritating to the skin and corrosive to the eye (undiluted), male Wistar rats were exposed to a 4-hour nose-only inhalation concentration of 0.05, 0.5, 1, and 5 mg/L (50, 500, 1,000, and 5,000 mg/m^3) with a MMAD 4.4, 2.9, 3.7, and 6.0 µm; GSD 2.7, 3, 4.2, and 2.9, respectively; 5 female rats were exposed to 1.1 or 5.5 mg/L with a MMAD 3.7 or 6.0 µm and GSD of 4.2 or 2.9, respectively [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14782</RecNum><DisplayText>[58, 59]</DisplayText><record><rec-number>14782</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596036284">14782</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sodium N-lauroylsarcosinate, CASRN: 137-16-6, EC number: 205-281-5, Eye Irritation</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/14123/7/4/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14783</RecNum><record><rec-number>14783</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596036540">14783</key></foreign-

keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Sodium N-lauroylsarcosinate, CASRN: 137-16-6, EC number: 205-281-5, Acute Toxicity: Inhalation</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/14123/7/3/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. The 5 mg/L dose resulted in fatality in all 10 animals tested within 1-2 h of dosing and the 0.5 mg/L dose resulted in fatality for 4/5 of the animals and exposure to 1 mg/L resulted in fatalities for the 10 animals within 1-2 days of exposure. Animals exposed to 0.05 mg/L did not demonstrate any adverse clinical signs or mortality at the conclusion of the study. At necropsy, red foci were noted on the lungs in animals receiving concentrations of ≥ 0.5 mg/L. The LC_{50} was reported to be 0.05-0.5 mg/L.

Repeated-dose inhalation studies were identified for oleoyl sarcosine, and dioctyl sodium sulfosuccinate (CASRN 577-11-7). Oleoyl sarcosine was evaluated in a 28-day nose-only inhalation study (6 hours/day, 5 days/week; Organization for Economic Cooperation and Development [OECD] Test Guideline 412) in male and female Fischer rats (5/group/sex) using concentrations of 0, 0.006, 0.02, or 0.06 mg/L (0, 6, 20, or 60 mg/m^3). The particle exposure MMAD was 1.11, 1.15, or 1.22 µm, GSD 1.68-2.57, and density 0.79 g/cm^2 for 6 hours/day, 5 days/week in 10% ethanol [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14784</RecNum

><DisplayText>[60]</DisplayText><record><rec-number>14784</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596036869">14784</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>N-methyl-N-[C18-(unsaturated)alkanoyl]glycine, CASRN: NA, EC number: 701-177-3, Repeated dose toxicity: Inhalation</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/21429/7/6/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]. Changes in the mean corpuscular volume (MCV), white blood cells (WBC), and lymphocytes were observed in male animals at the high exposure concentration. In female animals of the mid-concentration exposure group, reticulocyte counts were significantly reduced. Reflex bradypnea was noted in the animals at the mid and high concentrations, which is associated with severely irritating substances. All test concentrations caused effects at several sites of the respiratory tract with indications for local irritation, such as squamous metaplasia and epithelium proliferation and submucous acute inflammation at the base of the epiglottis. In the alveoli walls and bronchi, the most prominent finding was a focal early stage of fibrosis, but details were not provided at the dose level for this effect. Lung weights were increased at the highest dose. The LOAEC was 0.006 mg/L (6 mg/m^3) air in males and females; the basis for the effect level was local irritation.

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Dioctyl sulfosuccinate sodium salt (DOSS; CASRN 577-11-7) was evaluated in a 13-week inhalation study in male and female Sprague-Dawley rats (12/group/sex), exposed to an aerosol of a product containing 0.0042 mg/L (4.2 mg/m^3) DOSS, for 4 hours a day, 5 days a week (as reported in a secondary source; MMAD and GSD not reported) [ADDIN EN.CITE <EndNote><Cite><Author>CIR</Author><Year>2013</Year><RecNum>14785</RecNum><DisplayText>[61]</DisplayText><record><rec-number>14785</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596037107">14785</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>CIR</author></authors></contributors><titles><title>Safety Assessment of Alkyl Sulfosuccinate Salts as Used in Cosmetics, Re-Review, CIR Expert Panel Meeting, June 10-11, 2013</title><secondary-title>Cosmetic Ingredient Review (CIR), Washington, D.C.</secondary-title></titles><periodical><full-title>Cosmetic Ingredient Review (CIR), Washington, D.C.</full-title></periodical><pages>171, https://www.cir-safety.org/sites/default/files/Sulfosuccinates_RR.pdf</pages><dates><year>2013</year></dates><urls></urls></record></Cite></EndNote>]. There were no statistically significant differences in exposed and control groups for the mean body weight gain, survival, appearance and behavior, urinalysis values, and microscopic lesions. Significant differences were noted in the blood as indicated by elevated erythrocytic values (not otherwise specified) at 7 weeks and depressed mean corpuscular hemoglobin concentration values at 13 weeks in male rats. In females, depressed serum glutamic pyruvic transaminase and significant effect on absolute heart weight was observed at 7 weeks, depressed serum alkaline phosphatase was observed at 13 weeks and elevated glucose at 7 and 13-weeks. At 7 weeks, the lungs of necropsied animals showed

scattered foci of neutrophils and an increase in alveolar macrophages were reported in a single exposed male rat. A LOAEC of 4.2 mg/m^3 was identified based on the blood effects in male rats.

Mechanistic studies

Mechanistic studies on the pulmonary effects of anionic surfactants have been studied in dogs and/or sheep exposed to DOSS.

Increased minimum surface tension of lung extract or bronchioalveolar lavage fluid (BALF) was observed in dogs and sheep following *in vivo* aerosol exposure to DOSS in 1:1 mixture of ethanol and saline for 30 – 60 minutes, at a concentration that was selected to ensure a moderate degree of edema (estimated dose of 15 mg detergent/kg body weight) [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Anesthetized dogs were exposed *via* a ventilator to particle sizes of 0.5 to 15 μm with an MMAD of 3 μm. Light microscopic examination of the lungs 4 hours after exposure to DOSS aerosol observed no grossly destructive effects on alveolar cells or lung architecture in exposed dogs. However, a decrease in pulmonary compliance was observed that the authors hypothesized was due to an increase in surface tension in the alveoli in the presence of detergent.

Pulmonary clearance studies using radiolabeled aerosol tracers have evaluated whether detergent effects on the surfactant layer lead to increased alveolar permeability. Inhalation exposure to DOSS enhanced the pulmonary clearance of radiolabeled diethylenetriamine pentaacetic acid (DTPA), a relatively small hydrophilic molecule, indicating an increased alveolar permeability after detergent exposure [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In most studies,

this effect on alveolar permeability was seen in the absence of effects on blood gas levels or pulmonary compliance that occurs with higher exposure, indicating that the increase in alveolar permeability is a sensitive effect of detergent aerosol. The effect was demonstrated to be concentration-related in rabbits exposed to multiple dilutions (0.125, 0.25, 0.5, and 2%) with a MMAD of 1.7 μm of the liquid detergent [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Studies also evaluated the clearance of a radiolabeled aerosol of albumin, a much larger molecule, which was enhanced by DOSS as well, but to a lesser degree than DTPA [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Wang *et al.* (1993) [ADDIN EN.CITE ADDIN EN.CITE.DATA] observed an increase in protein flux from plasma to alveolar space after DOSS inhalation in sheep, which was attributed to disruption of the alveolar lining and increased microvascular permeability. The increased alveolar permeability observed in these studies was hypothesized to be a result of increased alveolar surface tension, which may result in increased permeability by opening previously closed pores (through which solutes pass) in the membrane or by stretching already open pores [ADDIN EN.CITE ADDIN EN.CITE.DATA]. However, as previously mentioned, surfactants can disrupt cell membranes; thus, this mechanism may be an alternate explanation [ADDIN EN.CITE <EndNote><Cite><Author>Burden</Author><Year>2012</Year><RecNum>14727</RecNum><DisplayText>[1]</DisplayText><record><rec-number>14727</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596017177">14727</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Burden, D.W.</author></authors></contributors><titles><title>Guide to the Disruption of Biological Samples - 2012, Version 1.1.</title><secondary-title>Random Primers</secondary-

title></titles><periodical><full-title>Random Primers</full-title></periodical><pages>1-25</pages><number>12</number><dates><year>2012</year></dates><urls></urls></record></Cite></EndNote>].

Cationic Surfactants

In vivo studies

Three acute inhalation toxicity studies were identified for cationic surfactants; one study each for DDAC, dioctadecyldimethylammonium chloride (DODMAC), and BAC (CASRN 8001-54-5). DDAC, which is corrosive to the skin and severely damaging to the eye [ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14786</RecNum><DisplayText>[69]</DisplayText><record><rec-number>14786</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596038295">14786</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration Dossier</author></authors></contributors><titles><title>Didecyldimethylammonium chloride, CASRN: 7173-51-5, EC number: 230-525-2, Skin irritation/corrosion</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/5864/7/4/2</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], was tested in rats (5/sex/dose, unspecified strain) exposed *via* inhalation to 0.05, 0.09, 0.13, 0.25, 1.36, or 4.54 mg/L (50, 90, 130, 250, 1,360, or 4,540 mg/m^3) for 2 hours with an observation period of 14 days (no additional exposure conditions reported). An LC_{50} of 0.07

mg/L was identified based on unspecified abnormalities identified in several organs including the lungs [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2016</Year><RecNum>14732</RecNum><DisplayText>[10]</DisplayText><record><rec-number>14732</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596018482">14732</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Subchronic Inhalation Toxicity Study of DDAC - Revised</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>25</pages><volume>HQ-OPP-2006-0338-0045</volume><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>] . A similar quaternary amine, DODMAC, which is irritating to the skin and causes serious damage to the eyes, was tested in albino rats (10 males, strain not specified) to the test substance (1:29 distilled water) *via* inhalation at 180 mg/L (180,000 mg/m^3) for one hour and observed for 14 days (no additional exposure conditions reported) [ADDIN EN.CITE <EndNote><Cite><Author>EURAR</Author><Year>2009</Year><RecNum>14787</RecNum><DisplayText>[70]</DisplayText><record><rec-number>14787</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596038841">14787</key></foreign-keys><ref-type name="Journal Article">17</ref-

type><contributors><authors><author>EURAR</author></authors></contributors><titles><titl e>European Union Risk Assessment Report (EURAR), CAS No: 107-64-2, EINECS No: 203-508-2, dimethyldioctadecylammonium chloride (DODMAC)</title><secondary-title>European Commission, Joint Research Centre, Institute for Health and Consumer Protection (IHCP), former Toxicology and Chemical Substances (TCS) European Chemicals Bureau (ECB)</secondary-title></titles><periodical><full-title>European Commission, Joint Research Centre, Institute for Health and Consumer Protection (IHCP), former Toxicology and Chemical Substances (TCS) European Chemicals Bureau (ECB)</full-title></periodical><pages>123, https://echa.europa.eu/documents/10162/46f2f114-12ff-4af4-8da7-72148b6a202e</pages><volume>14</volume><dates><year>2009</year></dates><urls></urls ></record></Cite></EndNote>]. No mortalities were reported and observed treatment-related clinical signs included preening, excessive masticatory (chewing) movements, excessive salivation stains, lacrimation, serosanguineous stains around the nose, and labored respiration. All animals appeared normal one day after dosing. The LD_{50} (1 h) was > 180 mg/L. BAC, which is corrosive to the skin and causes severe eye damage [ADDIN EN.CITE ADDIN EN.CITE.DATA], was tested in female Wistar rats (5/group) exposed *via* nose-only inhalation to 37.6 and 53 mg/m^3 for 4 hours and observed for 14 days or exposed to 30.6 mg/m^3 for 6 hours and BALF was measured 18 hours post-exposure (MMAD and GSD not reported) [ADDIN EN.CITE

<EndNote><Cite><Author>Swiercz</Author><Year>2008</Year><RecNum>14789</RecNum ><DisplayText>[72]</DisplayText><record><rec-number>14789</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596039305">14789</key></foreign-keys><ref-type name="Journal

Article">17</ref-type><contributors><authors><author>Swiercz, R.</author><author>Hałatek, T.</author><author>Wasowicz, W.</author><author>Kur, B.</author><author>Grzelińska, Z.</author><author>Majcherek, W.</author></authors></contributors><auth-address>Department of Toxicology and Carcinogenesis, Nofer Institute of Occupational Medicine, Łódź, Poland. radek@imp.lodz.pl</auth-address><titles><title>Pulmonary irritation after inhalation exposure to benzalkonium chloride in rats</title><secondary-title>Int J Occup Med Environ Health</secondary-title><alt-title>International journal of occupational medicine and environmental health</alt-title></titles><periodical><full-title>International journal of occupational medicine and environmental health</full-title><abbr-1>Int J Occup Med Environ Health</abbr-1></periodical><alt-periodical><full-title>International journal of occupational medicine and environmental health</full-title><abbr-1>Int J Occup Med Environ Health</abbr-1></alt-periodical><pages>157-63</pages><volume>21</volume><number>2</number><edition>2008/08/22</edition><keywords><keyword>Animals</keyword><keyword>Benzalkonium Compounds/administration & dosage/*toxicity</keyword><keyword>Female</keyword><keyword>Inhalation Exposure</keyword><keyword>Lung Diseases/*chemically induced/pathology</keyword><keyword>Organ Size/drug effects</keyword><keyword>Rats</keyword><keyword>Rats, Wistar</keyword></keywords><dates><year>2008</year></dates><isbn>1232-1087 (Print)
1232-1087</isbn><accession-num>18715840</accession-num><urls></urls><electronic-resource-num>10.2478/v10001-008-0020-1</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]. The LC_{50} was reported to

be approximately 53 mg/m^3 and BALF analysis reported increased inflammatory markers such as tumor necrosis factor (TNF)-a, interleukin (IL)-6. Indicators of lung damage, including increased LDH, total protein, and lung weight were also observed.

Three repeated dose inhalation studies of three different exposure durations were identified for DDAC: 14-day, 28-day, and 90-day.

In the 14-day study, male Sprague-Dawley rats were exposed *via* whole-body inhalation exposures to DDAC aerosols of 0.15 mg/m^3, 0.6 mg/m^3, and 3.6 mg/m^3 for 6 hours/day, 7 days/week [ADDIN EN.CITE <EndNote><Cite><Author>Lim</Author><Year>2014</Year><RecNum>14790</RecNum><DisplayText>[73]</DisplayText><record><rec-number>14790</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596039544">14790</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Lim, C. H.</author><author>Chung, Y. H.</author></authors></contributors><auth-address>Toxicity Research Team, Occupational Safety and Health Research Institute, KOSHA, Daejeon, Korea.</auth-address><titles><title>Effects of didecyldimethylammonium chloride on sprague-dawley rats after two weeks of inhalation exposure</title><secondary-title>Toxicol Res</secondary-title><alt-title>Toxicological research</alt-title></titles><periodical><full-title>Toxicol Res</full-title><abbr-1>Toxicological research</abbr-1></periodical><alt-periodical><full-title>Toxicol Res</full-title><abbr-1>Toxicological research</abbr-1></alt-periodical><pages>205-

10</pages><volume>30</volume><number>3</number><edition>2014/10/25</edition><keywords><keyword>Biocide</keyword><keyword>Didecyldimethylammonium chloride</keyword><keyword>Inhalation</keyword></keywords><dates><year>2014</year><pub-dates><date>Sep</date></pub-dates></dates><isbn>1976-8257 (Print)
1976-8257</isbn><accession-num>25343015</accession-num><urls></urls><custom2>PMC4206748</custom2><electronic-resource-num>10.5487/tr.2014.30.3.205</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]. The study authors reported an MMAD of 1.86 µm and a GSD of 2.75; however, individual values for each exposure concentration were not provided. Mild effects were noted in cell differential counts and cell damage parameters in BALF, in addition to inflammatory cell infiltration, and interstitial pneumonia at the medium and high exposures. The NOAEC was determined to be 0.15 mg/m^3.

In the intermediate exposure (4-week) study, male and female Sprague-Dawley rats (5 rats/sex/group) were exposed *via* dynamic nose-only inhalation to concentrations of 0, 0.08, 0.5, and 1.5 mg/m^3 DDAC (MMAD 1.4, 1.5, and1.9 µm, GSD 1.83, 1.86, and 1.87, density not reported) for 6 hours/day, 5 days/week [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2016</Year><RecNum>14732</RecNum><DisplayText>[10]</DisplayText><record><rec-number>14732</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596018482">14732</key></foreign-keys><ref-type name="Journal Article">17</ref-

type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Subchronic Inhalation Toxicity Study of DDAC - Revised</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>25</pages><volume>HQ-OPP-2006-0338-0045</volume><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>] . Body weights were significantly reduced in the high exposure group (males only) on days 14, 21, and 25. Lung weights were increased in females in the mid- and high-concentration groups and in males in the high concentration group. BALF analysis indicated that, at the high concentration, neutrophils and eosinophils increased with a concomitant decrease in macrophages. Histopathological findings in the nasal cavity were reported as minimal to mild with increased mucus of the respiratory epithelium in males and females at all exposures and ulceration of the nasal cavity observed in males and females in the high concentration group only. In males, there was an increase in cell count and total protein across all exposures. In females, there was an increase in LDH across all concentrations, but the small sample size precluded establishing statistical significance for the effects. A conservative LOAEC of 0.08 mg/m^3 was identified based on increased mucus of the respiratory epithelium and increased LDH; however, due to the mild effects and low number of animals/group, the effects were not statistically significant.

Commented [HT22]: Hyphens or not? This needs to be searched throughout; search for 'concentration' will find most...or maybe 'mid'

In the 13-week sub-chronic study, male and female Sprague-Dawley rats (10/group/sex) were exposed in whole body exposure chambers for 6 hours/day, 5 days/week [ADDIN EN.CITE

<EndNote><Cite><Author>Kim</Author><Year>2017</Year><RecNum>14736</RecNum><DisplayText>[74]</DisplayText><record><rec-number>14736</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596018905">14736</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kim, Y. S.</author><author>Lee, S. B.</author><author>Lim, C. H.</author></authors></contributors><auth-address>Chronic Inhalation Toxicity Research Center, Chemicals Toxicity Research Bureau, Occupational Safety and Health Research Institute, KOSHA, Daejeon, Korea.</auth-address><titles><title>Effects of Didecyldimethylammonium Chloride (DDAC) on Sprague-Dawley Rats after 13 Weeks of Inhalation Exposure</title><secondary-title>Toxicol Res</secondary-title><alt-title>Toxicological research</alt-title></titles><periodical><full-title>Toxicol Res</full-title><abbr-1>Toxicological research</abbr-1></periodical><alt-periodical><full-title>Toxicol Res</full-title><abbr-1>Toxicological research</abbr-1></alt-periodical><pages>7-14</pages><volume>33</volume><number>1</number><edition>2017/01/31</edition><keywords><keyword>Biocide</keyword><keyword>Didecyldimethylammonium chloride</keyword><keyword>Inhalation</keyword><keyword>Sub-chronic</keyword></keywords><dates><year>2017</year><pub-dates><date>Jan</date></pub-dates></dates><isbn>1976-8257 (Print)
1976-8257</isbn><accession-num>28133508</accession-num><urls></urls><custom2>PMC5266374</custom2><electronic-resource-num>10.5487/tr.2017.33.1.007</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]. The MMAD of the DDAC

aerosol was 0.63 µm, 0.81 µm, and 1.65 µm, and the geometric standard deviations were 1.62, 1.65, and 1.65 in the low (0.11 ± 0.06 mg/m^3), the middle (0.36 ± 0.20 mg/m^3) and the high (1.41 ± 0.71 mg/m^3) exposure groups, respectively. Body weight influenced by exposure to DDAC with the mean body weight approximately 35% lower in the high exposure (1.41 ± 0.71 mg/m^3) male group and 15% lower in the high exposure (1.41 ± 0.71 mg/m^3) female group compared to that of the control group. Albumin and LDH were unaffected in the BALF. Lung weight was increased in females in the mid- and high-concentration groups and in males in the high concentration group only, while inflammatory cell infiltration and interstitial pneumonia was observed in both the mid- and high-concentration groups. Tidal volume and minute volume were not significantly affected at any concentration. Severe histopathological symptoms such as proteinosis and/or fibrosis, were not reported. A NOAEC of 0.11 mg/m^3 was identified based on the increased lung weights in females and increase in inflammatory cells.

BAC was evaluated in a 2-week whole-body inhalation study in male and female Fischer rats (5/group/sex) to concentrations of 0.8, 4 and 20 mg/m^3 for 6 hours/day, 7 days/week [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Mean concentration of BAC in the whole-body exposure chambers of the T1 (0.8 mg/m^3), T2 (4 mg/m^3) and T3 (20 mg/m^3) groups during the exposure period was 0.84 ± 0.09, 4.01 ± 0.12, and 19.57 ± 0.97 mg/m^3, respectively; the MMAD of the aerosols was 1.614, 1.090, and 1.215 µm, respectively, and the GSD was 2.00, 1.86, and 1.51, respectively. The MMAD and GSD were confirmed to be within the range recommended by the OECD (OECD, 2018). Among the general signs observed during the exposure period, soiled perineal region, rales, and discharge were continuously observed during the 2-week recovery period.

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Exposure-related effects were observed in the upper airway. Nasal discharge, rale, and deep respiration were observed in the high concentration, and nasal discharge was observed in the low and mid concentrations. In the nasal cavity, ulceration with suppurative inflammation, squamous metaplasia, and erosion with necrosis were observed in the respiratory epithelium and transitional epithelium of the male and female high concentrations.

In the lower airways, degeneration and regeneration of terminal bronchiolar epithelium, smooth muscle hypertrophy of bronchioloalveolar junction, and cell debris in the alveolar lumens were observed in the mid and high concentration male groups and high concentration dose female group. Hypertrophy and hyperplasia of mucous cells in the bronchi or bronchiole were observed in both males and females. The squamous metaplasia of the respiratory epithelium and transitional epithelium, mucinous cell hypertrophy and proliferation of the respiratory epithelium, mucinous cell metaplasia of the transitional epithelium in the nasal cavities, and mucinous cell hypertrophy and proliferation of terminal bronchiole were considered adaptive changes after tissue injury. In the BALF analysis, the concentration of reactive oxygen species (ROS)/reactive nitrogen species (RNS), IL-1β, IL-6, and macrophage inflammatory protein (MIP)-2 decreased concentration-dependently at the end of the exposure period, which indicated oxidative damage, but did not show a concentration-dependent change at 4 weeks of recovery. The concentrations of TNF-α, IL-4, and transforming growth factor (TGF)-β did not show changes associated with test substance exposure. Relative lung weights were statistically significantly increased in males at the mid and high doses and in females at the high doses only. The study authors identified a LOAEC of 0.8 mg/m^3 based on effects in the nasal cavity.

Mechanistic studies

In vitro assays have demonstrated that cytotoxic effects of cationic surfactants have significantly greater toxicity to non-polarized than polarized mammalian cells [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In this study, cell viability as measured by LDH and MTT assays in non-polarized HeLa and fetal skin dendritic cells (FSDC) was more sensitive to the effects of different cationic surfactants of varying alkyl chain length and polar head groups than polarized cell lines Madin-Darby Canine Kidney (MDCK) and Caco-2. The cationic surfactant toxicity was shown to occur well below their CMC, and greater toxicity was observed with alkyl lengths of 10-12 than 14-16; however, this association was not strictly linear. In addition, the cationic surfactants with a larger polar head group (*i.e.*, benzalkonium) were 2-5 times more toxic than cationic surfactants with a more localized charge (*i.e.*, trimethylammonium).

The effects of BAC on cell viability, inflammatory response, and oxidative stress of human alveolar epithelial cells has been replicated *in vitro* using a dynamic culture condition that reflects the natural microenvironment of the lung to simulate the contraction and expansion of normal lungs [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Normal breathing levels were simulated (tidal volume 10%, 0.2Hz) through surface elongation of an elastic membrane in a dynamic culture system. This type of dynamic system provided easy control of exposure rate during the cell culture. The system assessed toxicity by culturing submerged cells with different BAC concentrations (0, 2, 5, 10, 20, and 40 µg/mL) under static and dynamic culture conditions. Following a 24-hr exposure to BAC, cellular metabolic activity, IL-8, and ROS levels were significantly affected, compared to untreated cells, when using either static or dynamic cell

growth conditions. The dynamic culture system, which more closely mimics lung conditions, showed a higher toxic response to BAC as indicated by increased ROS levels.

Dose-Response Analysis: Quantitative Points of Departure (PODs)

The animal inhalation toxicity data identified by the literature search and PODs from the studies are summarized in [REF _Ref46931035 \h * MERGEFORMAT]. All of the identified data are from animal studies and therefore need to be extrapolated to estimate the human inhalation exposure [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><DisplayText>[18]</DisplayText><record><rec-number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. The exposure duration adjustment and DAF approaches were described above. The

summary of RDDR inputs (*e.g.*, MMAD and GSD) and results are provided in [REF _Ref46931035 \h * MERGEFORMAT] for each of the toxicity studies from which PODs could be identified.

For the nonionic surfactant, octylphenoxypolyethoxyethanol, the effects observed (increased lung weights, alveolar/bronchiolar epithelial hyperplasia and lung inflammation) are consistent with lung effects in the thoracic region; therefore, the RDDR of 0.812 was used to calculate the HEC. For the anionic surfactant, oleoylsarcosine, the effects were seen in multiple regions of the respiratory tract, including squamous metaplasia and epithelium proliferation and submucous acute inflammation at the base of the epiglottis and early stages of fibrosis in the alveoli walls. Therefore, the total respiratory tract RDDR (0.970) was used to calculate the HEC. In the 28-day inhalation study with DDAC, effects were observed throughout the respiratory tract; therefore, the total respiratory tract RDDR (1.607) was used for calculating the HEC. Similarly, for the cationic surfactant, BAC histopathological cellular changes were observed in the nasal cavity and lungs, indicating the total respiratory tract RDDR (0.991) should be used to calculate the HEC. The RDDRs applied and HECs derived from the animal study PODs are provided in [REF _Ref46931035 \h * MERGEFORMAT].

Table [SEQ Table * ARABIC]. Inhalation Toxicity Points of Departure and Human Equivalent Concentrations (HEC) for Surfactants.

Surfactant Type	Chemical Substance	Inhalation Exposure Duration/Type	Study POD	Value (mg/m^3)	Reference	Density (g/cm^3) at 20 °C[1]	RDDR Model Input Parameters		RDDR[2]	HEC (mg/m^3)
							MMAD (μm)	GSD		
Nonionic	octylphenoxy polyethoxyethanol (CASRN 9002-93-1)	14-day, 6 hr/d, 5 d/wk; whole body	LOAEC	5.3	[ADDIN EN.CITE <EndNote><Cite><Author>MDEQ</Author><Year>2003</Year><RecNum>14731</RecNum><DisplayText>[8]</DisplayText><record><rec-number>14731</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0z	0.998 water vehicle	1.80	1.80	$RDDR_{ET}$ = 0.196 $RDDR_{TB}$ = 1.367 $RDDR_{PU}$ = 0.564 **$RDDR_{TH}$ = 0.812** $RDDR_{TOT}$ = 1.547	1.0 7.2 3.0 **4.4** 8.2

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					re0azr5ev earxfds0e rr5sr" timestamp ="159601 8112">14 731</key ></foreig n- keys><ref -type name="Jo urnal Article"> 17</ref- type><co ntributors ><authors ><author >MDEQ< /author>< /authors> </contribu tors><title s><title> To: Memo to File for Triton X- 100 (CAS # 9002- 93-1); From:					

					Gary Butterfield; Date: November 21,2003; Subject: Screening level for Triton X-100 (CAS # 9002-93-1)</title><secondary-title>Michigan Department of Environmental Quality (MDEQ)</secondary-title></titles><periodical><full-title>Michigan Department of					

					Environmental Quality (MDEQ)</full-title></periodical><pages>2</pages><dates><year>2003</year></dates><urls></urls></record></Cite></EndNote>]					
Anionic	oleoyl sarcosine (CASRN 110-25-8)	28-day, 6 hr/d, 5 d/wk; nose-only (OECD 412)	LOAEC	< 6	[ADDIN EN.CITE <EndNote><Cite><Author>Dossier</Author><Year>2020</Year><RecNum>14784</RecNum><DisplayText>[60]</DisplayText><recor	0.7893 ethanol vehicle	1.16	2.12	$RDDR_{ET}$ = 0.111 $RDDR_{TB}$ = 2.008 $RDDR_{PU}$ = 0.447 $RDDR_{TH}$ = 0.742 **$RDDR_{TOT}$ = 0.970**	< 0.6 < 12.0 < 2.7 < 4.5 **< 5.8**

[PAGE]

					d><rec-number>14784</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596036869">14784</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Registration					

					Dossier</author></authors></contributors><titles><title>N-methyl-N-[C18-(unsaturated)alkanoyl]glycine, CASRN: NA, EC number: 701-177-3, Repeated dose toxicity: Inhalation</title><secondary-title>European Chemicals Agency</secondary-title></titles><periodical><full-					

					title>European Chemicals Agency</full-title></periodical><pages>https://echa.europa.eu/hr/registration-dossier/-/registered-dossier/21429/7/6/3</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]					
Cationic	DDAC	4-week, 6 hr/d, 5 d/wk; nose-only	LOAEC[3] (lung effects)	0.08	[ADDIN EN.CITE <EndNote><Cite><Author>E	NR	1.60	1.85	$RDDR_{ET}$ = 0.211 $RDDR_{TB}$ = 1.674 $RDDR_{PU}$ = 0.539 $RDDR_{TH}$ = 0.854 **$RDDR_{TOT}$ = 1.607**	0.02 0.13 0.04 0.07 **0.13**

[PAGE]

					PA</Author><Year>2016</Year><RecNum>14732</RecNum><DisplayText>[10]</DisplayText><record><rec-number>14732</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5vearxfds0err5sr" timestamp="1596018482">14732</key></foreign-keys><ref					

					-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Subchronic Inhalation Toxicity Study of DDAC - Revised</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental					

					Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>25</pages><volume>HQ-OPP-2006-					

					0338-0045</volume><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>]					
	BAC	14-day, 6 hr/d, 7 d/wk; whole body	LOAEC (nasal effects)	0.8	[ADDIN EN.CITE ADDIN EN.CITE.DATA]	0.998 water vehicle 2% dose solution	1.31	1.79	$RDDR_{ET} = 0.106$ $RDDR_{TB} = 1.988$ $RDDR_{PU} = 0.528$ $RDDR_{TH} = 0.815$ **$RDDR_{TOT} = 0.991$**	0.08 1.59 0.42 0.65 **0.79**

MMAD: Mass Median Aerodynamic Diameter of inhalation study aerosol, average values listed; GSD: Geometric Standard Deviation of the inhalation study aerosol, average values listed; RDDR: Regional Deposited Dose Ration; ET: Extrathoracic; TB: Tracheobronchial; PU: Pulmonary; TH: Thoracic = TB + PU; TOT = ET + TB + PU.

[1]Exact density of administered compounds not reported (NR); vehicle density was listed when provided.

[2]RDDR values are for male and female animals, whichever was lower, as calculated using RDDR.exe and described in the Supporting Information file at "Section 2 RD

[3]conservative estimate: effects were not statistically significant.

NA: Data not available or RDDR values could not be calculated from the available information.

Commented [ST24]: SM comment: "Question: is this RDDR animal to worker (heavier breathing rate) or to general population?"

Commented [ST25R24]: I double checked this, the RDDR is based on ratios for minute volume, regional fractional deposition, and a normalizing factor (surface area for POE effects)

Benchmark Margin of Exposure Analysis

The substances shown in [REF _Ref46931035 \h * MERGEFORMAT] provide representative examples of PODs that may be applied to new chemistries that meet the Surfactant Criteria, after evaluating whether the chemical substances in [REF _Ref46931035 \h * MERGEFORMAT] are appropriate toxicological analogues for read-across to the new chemical substance. If a determination cannot be made on whether one of these chemical substances is an appropriate toxicological analogue, then the relevant substance from [REF _Ref46931035 \h * MERGEFORMAT] should be identified as a comparator substance[3] for use in the Tiered-Testing Strategy, discussed below. Though the initial starting point for deriving a benchmark MOE is based on a composite of the default values of 10 for each of the individual values for UF_H, UF_A, and UF_L, refinements may be warranted based on dosimetric adjustments to the applied concentrations used for establishing the experimental PODs. As shown in [REF _Ref46931035 \h * MERGEFORMAT], the data-derived uncertainty factors were based on RDDRs that were used as DAFs to account for animal-to-human toxicokinetic differences.

In the case of surface-active substances meeting the Surfactant Criteria, EPA has recently adopted a generalized approach that has historically been applied on a case-by-case basis for chemical substances, in recognition that surface-active effects that lead to irritation/corrosion do

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[3] A comparator substance is one that may possess similar properties to the new chemical substance and for which inhalation toxicity data are available. EPA may "read-across" the toxicity data from the comparator substance to the new chemical substance when no other information is available. The tiered-testing approach for this category is designed to determine whether this practice may be refined or supported by additional data. As such, the comparator substance should be used in side-by-side testing in Tiers I-III with a new chemical substance to aid with interpreting the test results of the new chemical substance.

not require absorption, metabolism, distribution, or elimination (ADME) (See, *e.g.*, EPA, 2020 [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2020</Year><RecNum>14794</RecNum><DisplayText>[78]</DisplayText><record><rec-number>14794</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596040494">14794</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Hazard Characterization of Isothiazolinones in Support of FIFRA Registration Review</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>84, https://www.regulations.gov/contentStreamer?documentId=EPA-HQ-OPP-2013-0605-0051&contentType=pdf</pages><volume>EPA-HQ-OPP-2013-0605-0051</volume><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]). In the context of this publication, irritation/corrosion include those effects in the respiratory tract that lead to inflammation, hyperplasia, and metaplasia. For chemical substances that act *via* a surface-active adverse outcome pathway (AOP) [ADDIN EN.CITE <EndNote><Cite><Author>Sorli</Author><Year>2020</Year><RecNum>14800</RecNum><DisplayText>[79]</DisplayText><record><rec-number>14800</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041625">14800</key></foreign-keys><ref-type name="Journal

Article">17</ref-type><contributors><authors><author>Sorli, J. B.</author></authors></contributors><titles><title>Lung Surfactant Function Disruption Leading to Acute Inhalation Toxicity</title><secondary-title>AOPWiki</secondary-title></titles><periodical><full-title>AOPWiki</full-title></periodical><pages>https://aopwiki.org/aops/302</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], the default values for UF_H and UF_A are reduced to 3 (*i.e.*, $10^{0.5}$ or 3.162) to account for the uncertainty/variability for toxicodynamics, whereas the toxicokinetic component is reduced to 1. In order to apply these reductions, the following criteria must be established:

1. A description of the AOP,
2. A discussion of why the AOP is unlikely or likely to differ between humans, in the case of UF_H, or between animals in comparison to humans, in the case of UF_A, and
3. A discussion as to why the ADME of the chemical substance is addressed by the use of dosimetry modeling.

When the above criteria are met, application of the appropriate DAF (*e.g.*, the RDDR for particles) should still be applied, given that deposition is the most appropriate dose metric for assessing acute/subacute effects from surface-active agents. However, since the DAF accounts for the toxicokinetic component of UF_A, the remaining value of 3 (*i.e.*, $10^{0.5}$ or 3.16) should be retained for the toxicodynamics component of the UF_A.

Based on these information and criteria, the following composite values are appropriate to describe intra- and interspecies variability (*i.e.*, $UF_H \times UF_A$):

UF_H = 10 or 3: The default value of 10 should be applied when the available information does not support each of the above criteria. If the available information supports all three of the above criteria, then a value of 3 may be applied. The reduced value represents a reduction in the toxicokinetic component of this UF to 1, with the remaining value of 3 accounting for the toxicodynamic component.

UF_A = 10 or 3: The default value of 10 should be applied when the available information does not support the application of dosimetric adjustments for quantifying an HEC or when the available information does not support each of the above three criteria. If the available information allows derivation of an HEC and/or application of the above criteria, then a value of 3 may be applied, which represents a reduction in the toxicokinetic component to 1 and application of a value of 3 for toxicodynamics.

UF_L = 10 or 1: If the POD from the experimental study is based on a LOAEC, then a default value of 10 should be applied, unless there is information to support that a reduced value is warranted. If the experimental data are amenable to benchmark dose modeling, a BMCL should be calculated and a value of 1 should be applied for this area of uncertainty.

The above considerations and approaches support the application of a benchmark MOE ranging from 10 (*i.e.*, $10^{0.5} \times 10^{0.5} \approx 10$) to 1,000 depending on the chemical substance identified as an

appropriate toxicological analogue and available data on the new chemical substance. In those instances where the data are too limited to determine when one of the chemical substances is appropriate for extrapolating the hazards to the new chemical substance, experimental testing should be performed to aid with informing the quantitative assessment, as discussed under the Tiered-Testing Strategy.

Uncertainties and Limitations

The assessment framework outlined includes a number of uncertainties and limitations, including those associated with extrapolating the hazards identified from the chemical substances shown in [REF _Ref46931035 \h * MERGEFORMAT]. Uncertainties associated with using animal studies to estimate human toxicity are recognized and methods are presented to reduce extrapolation uncertainties [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2014</Year><RecNum>14795</RecNum><DisplayText>[80]</DisplayText><record><rec-number>14795</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596040729">14795</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Guidance on Grouping of Chemicals, Second Edition, Series on Testing & Assessment</title><secondary-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</secondary-title></titles><periodical><full-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working

Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</full-title></periodical><pages>141, http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2014)4&doclanguage=en</pages><volume>ENV/JN/MONO(2014)4</volume><dates><year>2014</year></dates><urls></urls></record></Cite></EndNote>]. Procedures for the adjustment of exposure durations for inhalation exposures and application of DAFs to derive HECs are well-established procedures for reducing uncertainties associated with the toxicokinetic aspects of animal-to-human extrapolation factors and derivation of benchmark MOEs (*i.e.*, type and magnitude of uncertainty factors) [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2002</Year><RecNum>14743</RecNum><DisplayText>[17, 18]</DisplayText><record><rec-number>14743</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019884">14743</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>A Review of the Reference Dose and Reference Concentration Processes</title><secondary-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Risk Assessment Forum, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>192, https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf</pages><volume>EPA/630/P-02/002F</volume><dates><year>2002</year></dates><urls></urls></record></Cite><Cite><Author>EPA</Author><Year>1994</Year><RecNum>14746</RecNum><record><rec-

number>14746</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596021628">14746</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry</title><secondary-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</secondary-title></titles><periodical><full-title>Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC</full-title></periodical><pages>389, https://www.epa.gov/sites/production/files/2014-11/documents/rfc_methodology.pdf</pages><volume>EPA/600/8-90/066F</volume><dates><year>1994</year></dates><urls></urls></record></Cite></EndNote>]. Likewise, EPA has recommended that BMD modeling be employed whenever possible to identify a POD and to reduce uncertainties associated with using a LOAEL from a toxicity study.

Given the small number of chemical substances that meet the Surfactant Criteria that have concentration-response inhalation toxicity data, the applicability of the chemical substances in [REF _Ref46931035 \h * MERGEFORMAT] to new chemical substances needs to be carefully considered, with attention given to the influence of additional functional groups on the toxicity of the new chemical substance. Risk assessors should consider the surface tension and CMC criteria ([REF _Ref47613375 \h * MERGEFORMAT]) compared to these measurements for the new chemical substance and the influence of the presence or absence of additional functional groups on these criteria (*e.g.*, would a particular functional group increase or decrease toxicity,

for example by another mechanism of action). If such structural differences are judged not to significantly influence properties and toxicity, such that the new chemical substance is expected to have comparable or lower toxicity, the hazard(s) and risk(s) should be characterized using the chemical substance as a toxicological analogue to the new chemical substance. Of course, uncertainties regarding this extrapolation should be acknowledged in the risk characterization.

For instances where the notifier of the new chemical substance and/or EPA is unable to conclude that one of the chemical substances in [REF _Ref46931035 \h * MERGEFORMAT] is comparable to or represents an acceptable toxicological analogue to the new chemical substance, then the Tiered-Testing Strategy provided could be used to determine whether the new chemical substance has lower, comparable, or higher toxicity to the relevant chemical substance in [REF _Ref46931035 \h * MERGEFORMAT], as a comparator substance and not as a toxicological analogue. Prior to conducting such testing, the scientific basis for selecting the comparator substance to the new chemical substance should be understood and a rationale provided as to why the comparator substance will be used for testing.

Use of New Approach Methods (NAMs) and *In Vitro* Testing Strategies to Reduce or Replace Vertebrate Testing

The amended TSCA requires EPA to reduce reliance on animal testing using methods and strategies that "provide information of equivalent or better scientific quality and relevance for assessing risks of injury to health or the environment" [ADDIN EN.CITE <EndNote><Cite><Author>U.S.C.</Author><Year>2016</Year><RecNum>14796</RecNum><DisplayText>[81]</DisplayText><record><rec-number>14796</rec-number><foreign-

keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041048">14796</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>U.S.C.</author></authors></contributors><titles><title>Title 15-Commerce and Trade, Chapter 53-Toxic Substances Control, Subchapter I-Control of Toxic Substances</title><secondary-title>United States Code (U.S.C.)</secondary-title></titles><periodical><full-title>United States Code (U.S.C.)</full-title></periodical><pages>https://uscode.house.gov/view.xhtml?path=/prelim@title15/chapter53&edition=prelim</pages><dates><year>2016</year></dates><urls></urls></record></Cite></EndNote>]. Moreover, the amended TSCA requires entities undertaking voluntary testing for submission to EPA to first "…attempt to develop the information by means of an alternative test method or strategy …before conducting new vertebrate testing…" [ADDIN EN.CITE <EndNote><Cite><Author>U.S.C.</Author><Year>2016</Year><RecNum>14796</RecNum><DisplayText>[81]</DisplayText><record><rec-number>14796</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041048">14796</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>U.S.C.</author></authors></contributors><titles><title>Title 15-Commerce and Trade, Chapter 53-Toxic Substances Control, Subchapter I-Control of Toxic Substances</title><secondary-title>United States Code (U.S.C.)</secondary-title></titles><periodical><full-title>United States Code (U.S.C.)</full-title></periodical><pages>https://uscode.house.gov/view.xhtml?path=/prelim@title15/chapter53&edition=prelim</pages><dates><year>2016</year></dates><urls></urls></record></Cit

e></EndNote>]. Additionally, in 2019, EPA was directed to prioritize efforts to use NAMs to reduce animal testing [ADDIN EN.CITE <EndNote><Cite><Author>Wheeler</Author><Year>2019</Year><RecNum>14797</RecNum><DisplayText>[82]</DisplayText><record><rec-number>14797</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041176">14797</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Wheeler, A.R.</author></authors></contributors><titles><title>Directive to Prioritize Effects to Reduce Animal Testing</title><secondary-title>United States Environmental Protection Agency</secondary-title></titles><periodical><full-title>United States Environmental Protection Agency</full-title></periodical><pages>3, https://www.epa.gov/sites/production/files/2019-09/documents/image2019-09-09-231249.pdf</pages><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>]. Multiple NAMs exist which can be used to assess hazards and risks of new chemical substances that meet the Surfactant Criteria, including validated OECD methods for *in vitro* irritation testing and *in vitro* methods to specifically assess respiratory toxicity. Several methods are described within a tiered-testing strategy recognizing that these assays are provided as examples and the development of NAMs is advancing rapidly. As such, the NAMs included here should not be considered all-inclusive or a final compilation. EPA strongly encourages the development and use of NAMs, particularly to reduce or replace the use of vertebrate animals and is open to considering and discussing additional NAMs with PMN submitters during a pre-notice consultation [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2020</Year><RecNum>14829</RecNum><

DisplayText>[83]</DisplayText><record><rec-number>14829</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596098792">14829</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Schedule a Pre-Submission Meeting, Reviewing New Chemicals under the Toxic Substances Control Act (TSCA)</title><secondary-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>https://www.epa.gov/reviewing-new-chemicals-under-toxic-substances-control-act-tsca/forms/program-contacts-and</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>].

In the interest of reducing or replacing vertebrate testing and designing a scientifically robust testing approach, when a surfactant is determined to be respirable, EPA encourages evaluating its potential to cause pulmonary toxicity using an AOP approach. The OECD provides "An AOP is an analytical construct that describes a sequential chain of causally linked events at different levels of biological organization that lead to an adverse health or ecotoxicological effect" and that "AOPs are the central element of a toxicological knowledge framework being built to support chemical risk assessment based on mechanistic reasoning" [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2020</Year><RecNum>14798</RecNum><DisplayText>[84]</DisplayText><record><rec-number>14798</rec-number><foreign-

keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041285">14798</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Adverse Outcome Pathways, Molecular Screening and Toxicogenomics</title><secondary-title>Organization for Economic Cooperation and Development (OECD)</secondary-title></titles><periodical><full-title>Organization for Economic Cooperation and Development (OECD)</full-title></periodical><pages>http://www.oecd.org/env/ehs/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>].

Representative key elements of AOPs are the molecular initiating events (MIEs), cellular level events (CLEs), organ or tissue level events (OLEs), and organism consequent events (OCEs). For surfactants, the initial key event is proposed to be the interaction of the substance with epithelial lining fluid or lung-surfactant (MIE) and/or the molecular interaction of the substance itself with cell membranes of the epithelium in the respiratory tract (MIE), resulting in the disruption of lung cells due to loss of lung cell surfactant function (CLE) and/or the loss of membrane integrity (CLE). These initial events may lead to different OLEs (*e.g.*, cytotoxicity and perturbation of airway epithelial cells, alveolar collapse, loss of barrier function, blood extravasation, and impaired oxygenation of blood), which may finally lead to organism consequences (OCE) (*e.g.*, pneumonia, limited lung function by chronic obstruction (COPD),

fibroses, *etc.*). AOPs in various stages of development are useful for different purposes and an AOP may still be useful even if it has not been formally evaluated by the OECD.

An AOP can be used to help design a testing strategy and to identify NAMs that can query the key events leading up to the adverse outcome. As an example, using the respiratory irritant chlorothalonil, Syngenta Crop Protection applied a NAM for the assessment of inhalation toxicology based on AOP [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The approach involved derivation of the POD from an *in vitro* assay and the integration of the *in vitro* POD for calculation of HECs for the inhalation risk assessment. Similar approaches can be used for surfactants where *in vitro* systems may be used to investigate specific key events in the AOP and confirm that a new chemical substance fits within the boundaries of the Surfactant Category or a sub-category and therefore may act like a surfactant (group assignment *via* similar AOP) and/or if other substance specific properties lead to a predominant type of key event within the AOP. Further, *in vitro* tests may deliver information while avoiding *in vivo* testing or provide helpful information on dose-selection for *in vivo* testing, if needed. Some *in vitro* tests, such as by capillary surfactometer, may be useful in preliminary screening of chemicals to be tested, but do not by themselves constitute adequate tests for acute pulmonary effects of these chemicals. This information should be taken into consideration within the design of additional tests. These assays can be used as part of a weight of scientific evidence evaluation to determine whether animal testing is needed or if a POD can be determined for risk assessment purposes without the use of animals. These tests may also provide insight on one or more components of the AOP.

Based on the surfactant AOP framework under development [ADDIN EN.CITE <EndNote><Cite><Author>Sorli</Author><Year>2020</Year><RecNum>14800</RecNum><DisplayText>[79]</DisplayText><record><rec-number>14800</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596041625">14800</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sorli, J. B.</author></authors></contributors><titles><title>Lung Surfactant Function Disruption Leading to Acute Inhalation Toxicity</title><secondary-title>AOPWiki</secondary-title></titles><periodical><full-title>AOPWiki</full-title></periodical><pages>https://aopwiki.org/aops/302</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>], a number of different types of *in vitro* test methods, summarized in [REF _Ref46931271 \h * MERGEFORMAT], may provide potentially useful information for informing the various elements of the surfactant AOP. Clippinger *et al*. (2018) [ADDIN EN.CITE ADDIN EN.CITE.DATA] have also described a decision tree and potential MIEs and key events that can be used to design pathway-based approaches for *in vitro* testing of acute inhalation exposures.

Table [SEQ Table * ARABIC]. *In Vitro* Test Methods and New Approach Methods That May Be Useful for Evaluating Chemicals for Inclusion in Surfactant AOP and Category.

Surfactant AOP	*Information on AOP*	*In Vitro* Assay	Test System
Molecular Initiating Events (MIEs)	MIE for interaction with pulmonary surfactant/loss of function	*In Vitro* Respiratory Toxicity Assays	• *In vitro* lung surfactant interaction, *e.g.*, as described by Sorli *et al.* (2018) [ADDIN EN.CITE ADDIN EN.CITE.DATA]
	MIE for disruption of cell membrane components and interaction /penetration through cell membrane	*Hemoglobin Denaturation Assay, Liposome Assay, and In Vitro/Ex Vivo* Irritation Assays	• Hemoglobin denaturation assay, *e.g.*, as described by Hayashi *et al.* (1994) [ADDIN EN.CITE <EndNote><Cite><Author>Hayashi</Author><Year>1994</Year><RecNum>14838</RecNum><DisplayText>[89]</DisplayText><rec id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596732926">14838</key></foreign-keys><ref-type name="Journal Article"> H.</author><author>Fukuda, T.</author><author>Tamura, U.</author><author>Kato, S.</author></authors></contributors><auth-addre address><titles><title>Multivariate factorial analysis of data obtained in seven in vitro test systems for predicting eye irritancy</title><sec published in association with BIBRA</alt-title></titles><periodical><full-title>Toxicology in vitro : an international journal published in periodical><full-title>Toxicology in vitro : an international journal published in association with BIBRA</full-title><abbr-1>Toxicol In V 20</pages><volume>8</volume><number>2</number><edition>1994/04/01</edition><dates><year>1994</year><pub-dates><date>Ap num>20692908</accession-num><urls></urls><electronic-resource-num>10.1016/0887-2333(94)90185-6</electronic-resource-num><re provider><language>eng</language></record></Cite></EndNote>]. • Liposome assay, *e.g.*, as described by Kapoor *et al.* (2009) [ADDIN EN.CITE <EndNote><Cite><Author>Kapoor</Author><Year>2009 number>14834</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596539300"> type><contributors><authors><author>Kapoor, Y.</author><author>Howell, B. A.</author><author>Chauhan, A.</author></authors></ Florida 32611, USA.</auth-address><titles><title>Liposome assay for evaluating ocular toxicity of surfactants</title><secondary-title>In science</alt-title></titles><periodical><full-title>Investigative ophthalmology & visual science</full-title><abbr-1>Invest Ophthalm science</full-title><abbr-1>Invest Ophthalmol Vis Sci</abbr-1></alt-periodical><pages>2727-35</pages><volume>50</volume><numb Diseases/chemically induced</keyword><keyword>Corneal Diseases/chemically induced</keyword><keyword>*Diagnostic Techniques Ophthalmological</keyword><keyword>Fluoresceins/*metabolism</keyword><keyword>Fluorescent Dyes/*metabolism</keyword><ke Measurements</keyword><keyword>Models, Theoretical</keyword><keyword>Permeability/drug effects</keyword><keyword>Surfac dates><date>Jun</date></pub-dates></dates><isbn>0146-0404</isbn><accession-num>19168898</accession-num><urls></urls><electr provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>].

			• OECD *In vitro/ex vivo* eye irritation tests for penetrance, *e.g.*, Reconstructed human Cornea-like Epithelium (RhCE) (OECD 492) [ADDI <EndNote><Cite><Author>OECD</Author><Year>2019</Year><RecNum>14803</RecNum><DisplayText>[91]</DisplayText><reco id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596043912">14803</key></foreign-keys><ref-type name="Journal Article"> type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Reconstructed human Cornea-like Epithe irritation or serious eye damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><period https://www.oecd-ilibrary.org/docserver/9789264242548- en.pdf?expires=1596044765&id=id&accname=guest&checksum=C972EFF7A2459C37BF048A1BDC82F2D4</pages><v Bovine Corneal Opacity and Permeability Test (OECD 437) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>202 number>14802</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596043719"> type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Bovine Corneal Opacity And Permeabili Requiring Classification For Eye Irritation Or Serious Eye Damage</title><secondary-title>OECD Guidelines for the Testing of Chemica Chemicals</full-title></periodical><pages>28, https://www.oecd-ilibrary.org/docserver/9789264203846- en.pdf?expires=1596044549&id=id&accname=guest&checksum=6B06BCD6D113D26A04C508907C001D91</pages><v Isolated Chicken Eye Test (OECD 438) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044057">14804</key></forei type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Isolated chicken eye test method for iden irritation or serious eye damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><period https://www.oecd-ilibrary.org/docserver/9789264203860- en.pdf?expires=1596044906&id=id&accname=guest&checksum=37A7598040CEC8996E712477F0A603D7</pages><vol *etc*.
Cellular Level Events (CLEs)	CLE for loss of membrane integrity/general cytotoxicity	*In Vitro/Ex Vivo* Cytotoxicity Assays	• OECD *In vitro/ex vivo* eye irritation tests for cytotoxicity, *e.g.*, Reconstructed human Cornea-like Epithelium (RhCE) (OECD 492) [ADD <EndNote><Cite><Author>OECD</Author><Year>2019</Year><RecNum>14803</RecNum><DisplayText>[91]</DisplayText><reco id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596043912">14803</key></foreign-keys><ref-type name="Journal Article"> type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Reconstructed human Cornea-like Epithe irritation or serious eye damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><period https://www.oecd-ilibrary.org/docserver/9789264242548- en.pdf?expires=1596044765&id=id&accname=guest&checksum=C972EFF7A2459C37BF048A1BDC82F2D4</pages><v Bovine Corneal Opacity and Permeability Test (OECD 437) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>202 number>14802</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596043719"> type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Bovine Corneal Opacity And Permeabili Requiring Classification For Eye Irritation Or Serious Eye Damage</title><secondary-title>OECD Guidelines for the Testing of Chemica Chemicals</full-title></periodical><pages>28, https://www.oecd-ilibrary.org/docserver/9789264203846- en.pdf?expires=1596044549&id=id&accname=guest&checksum=6B06BCD6D113D26A04C508907C001D91</pages><v

			Isolated Chicken Eye Test (OECD 438) [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044057">14804</key></forei type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Isolated chicken eye test method for iden irritation or serious eye damage</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><period https://www.oecd-ilibrary.org/docserver/9789264203860-en.pdf?expires=1596044906&id=id&accname=guest&checksum=37A7598040CEC8996E712477F0A603D7</pages><vol *etc*.
			• Cell membrane integrity test (LDH-cytotoxicity assay), MTT assay, TEER, ATP, or lysosomal membrane integrity test. • BALB/c3T3/A549 lung cells neutral red uptake (NRU) cytotoxicity test, a test for basal cytotoxicity (ICCVAM, 2006) [ADDIN EN.CITE <EndNote><Cite><Author>ICCVAM</Author><Year>2006</Year><RecNum>14805</RecNum><DisplayText>[94]</DisplayText><r id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044231">14805</key></foreign-keys><ref-type name="Journal Article"> type><contributors><authors><author>ICCVAM</author></authors></contributors><titles><title>In vitro Cytotoxicity Test Methods fo title>ICCVAM Test Method Evaluation Report</secondary-title></titles><periodical><full-title>ICCVAM Test Method Evaluation Repo https://ntp.niehs.nih.gov/iccvam/docs/acutetox_docs/brd_tmer/at-tmer-complete.pdf</pages><volume>NIH Publication No. 07-4519</vol
Organ or Tissue Level Events (OLEs)	OLE for tissue level events	Human organotypic Airway Epithelial Cultures	• 3D constructs of human-derived cell cultures of differentiated airway epithelial cells (*e.g.*, EpiAirway™, MucilAir™, SmallAir™, EpiAlve
	OLE for tissue level events	Specific *Ex Vivo* Respiratory Toxicity Assays	• Precision-cut lung slice test, *e.g.,* as described by Hess *et al.* (2016) [ADDIN EN.CITE ADDIN EN.CITE.DATA] and Neuhaus *et a*

MIEs

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The surfactant AOP is hypothesized to consist of two MIEs that may be informed by *in vitro* assays to determine whether a particular chemistry causes adverse effects on the epithelial lining fluid (ELF) or pulmonary surfactant system (MIE #1) or cytotoxicity to airway epithelial or pulmonary cell membranes (MIE #2), or both. For MIE #1, Sorli *et al.* (2017) [ADDIN EN.CITE ADDIN EN.CITE.DATA] developed an *in vitro* lung surfactant interaction assay that specifically measures whether the substance interferes with lung surfactant function. The assay was initially developed for predicting the effect of waterproofing agents that were shown to be acutely toxic to mice. The authors noted that it may be overly conservative for some substances. Nevertheless, this assay investigated a basic principle (*e.g.*, MIE #1) which may also be relevant for some types of surfactants. For MIE #2, the hemoglobin denaturation and liposome assays and *in vitro* eye irritation assays do not directly measure effects on membranes of pulmonary cells; however, these assays have been shown to provide indirect lines of evidence as a screening approach for determining the ability of surfactants to interact with cellular membrane components and cell membrane penetration. For example, Hayashi *et al.* (1995) [ADDIN EN.CITE <EndNote><Cite><Author>Hayashi</Author><Year>1995</Year><RecNum>14833</RecNum><DisplayText>[98]</DisplayText><record><rec-number>14833</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596539110">14833</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hayashi, T.</author><author>Itagaki, H.</author><author>Fukuda, T.</author><author>Tamura, U.</author><author>Sato,

Y.</author><author>Suzuki, Y.</author></authors></contributors><auth-address>Shiseido Research Center, Yokohama, Japan.</auth-address><titles><title>Hemoglobin denaturation caused by surfactants</title><secondary-title>Biol Pharm Bull</secondary-title><alt-title>Biological & pharmaceutical bulletin</alt-title></titles><alt-periodical><full-title>Biological & Pharmaceutical Bulletin</full-title><abbr-1>Biol. Pharm. Bull.</abbr-1></alt-periodical><pages>540-3</pages><volume>18</volume><number>4</number><edition>1995/04/01</edition><keywords><keyword>Chromatography, High Pressure Liquid</keyword><keyword>Circular Dichroism</keyword><keyword>Hemoglobins/*chemistry</keyword><keyword>Irritants/pharmacology</keyword><keyword>Protein Denaturation/drug effects</keyword><keyword>Sodium Dodecyl Sulfate/pharmacology</keyword><keyword>Spectrophotometry</keyword><keyword>Structure-Activity Relationship</keyword><keyword>Surface-Active Agents/*pharmacology</keyword><keyword>Taurine/analogs & derivatives/pharmacology</keyword></keywords><dates><year>1995</year><pub-dates><date>Apr</date></pub-dates></dates><isbn>0918-6158 (Print)
0918-6158</isbn><accession-num>7655423</accession-num><urls></urls><electronic-resource-num>10.1248/bpb.18.540</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>] showed that charged surfactant molecules can interfere with charged side chains of the hemoglobin protein. These interactions lead to disruption of the three-dimensional (3D) structure of hemoglobin, causing a change in light absorbance that can be measured. Increasing concentrations of SDS and sodium

lauroylmethyltaurate (LMT) were tested in this assay and showed concentration dependent increases in hemoglobin denaturation, which correlated with irritation effects in the Draize eye test [ADDIN EN.CITE ADDIN EN.CITE.DATA].

The liposome assay can be used to assess disruption of the lipid bilayer of the membrane from interaction with surfactant chemistries. Kapoor *et al*. (2009) [ADDIN EN.CITE <EndNote><Cite><Author>Kapoor</Author><Year>2009</Year><RecNum>14834</RecNum><DisplayText>[90]</DisplayText><record><rec-number>14834</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596539300">14834</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kapoor, Y.</author><author>Howell, B. A.</author><author>Chauhan, A.</author></authors></contributors><auth-address>Department of Chemical Engineering, University of Florida, Gainesville, Florida 32611, USA.</auth-address><titles><title>Liposome assay for evaluating ocular toxicity of surfactants</title><secondary-title>Invest Ophthalmol Vis Sci</secondary-title><alt-title>Investigative ophthalmology & visual science</alt-title></titles><periodical><full-title>Investigative ophthalmology & visual science</full-title><abbr-1>Invest Ophthalmol Vis Sci</abbr-1></periodical><alt-periodical><full-title>Investigative ophthalmology & visual science</full-title><abbr-1>Invest Ophthalmol Vis Sci</abbr-1></alt-periodical><pages>2727-35</pages><volume>50</volume><number>6</number><edition>2009/01/27</edition><keywords><keyword>Conjunctival Diseases/chemically induced</keyword><keyword>Corneal Diseases/chemically induced</keyword><keyword>*Diagnostic Techniques,

Ophthalmological</keyword><keyword>Fluoresceins/*metabolism</keyword><keyword>Fluor
escent
Dyes/*metabolism</keyword><keyword>Humans</keyword><keyword>*Liposomes</keywor
d><keyword>Luminescent Measurements</keyword><keyword>Models,
Theoretical</keyword><keyword>Permeability/drug effects</keyword><keyword>Surface-
Active Agents/*toxicity</keyword></keywords><dates><year>2009</year><pub-
dates><date>Jun</date></pub-dates></dates><isbn>0146-0404</isbn><accession-
num>19168898</accession-num><urls></urls><electronic-resource-num>10.1167/iovs.08-
2980</electronic-resource-num><remote-database-provider>NLM</remote-database-
provider><language>eng</language></record></Cite></EndNote>] measured the release of calcein dye from liposomes following exposure to various surfactants and showed a positive correlation with these findings and data from the Draize eye test. The hemoglobin denaturation and liposomal assays were both optimized and validated against eye irritation data; therefore, these assays may provide an opportunity to evaluate the effects of surfactants on the respiratory tract. Further *in vitro* testing of known surfactants with existing data alongside new chemical substances will help benchmark the results. Nonetheless, these assays are envisioned to be useful for understanding the potential for a new surfactant substance to act *via* MIE #2 in the respiratory tract.

The use of *ex vivo* eye irritation studies may provide indirect measures of surfactants on cell membranes, which may be relevant to the effects observed from comparator substances in the respiratory tract. For example, Bader *et al*. (2013) [ADDIN EN.CITE
<EndNote><Cite><Author>Bader</Author><Year>2014</Year><RecNum>14807</RecNum>

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<DisplayText>[100]</DisplayText><record><rec-number>14807</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044694">14807</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bader, J.E.</author><author>Norman, K.G.</author><author>Raabe, H.</author></authors></contributors><titles><title>Predicting Ocular Irritation of Surfactants Using the Bovine Corneal Opacity and Permeability Assay</title><secondary-title>Insitute for In Vitro Sciences, Inc., Gaithersburg, M.D.</secondary-title></titles><periodical><full-title>Insitute for In Vitro Sciences, Inc., Gaithersburg, M.D.</full-title></periodical><pages>https://iivs.org/wp-content/uploads/2018/08/iivs_poster_predicting-ocular-irritation-of-surfactants-using-the-bovine-corneal-opacity-and-permeability-assay.pdf</pages><dates><year>2014</year></dates><urls></urls></record></Cite></EndNote>] reported that the Bovine Corneal Opacity and Permeability (BCOP) assay was effective at demonstrating that nonionic (*i.e.*, octylphenoxypolyethoxyethanol), anionic (*i.e.*, SDS), and cationic (*i.e.*, BAC) substances cause irritation to the eye; however, the authors also noted that the endpoints evaluated in this assay should be carefully assessed independently. The permeability score was more predictive of eye irritation than the ocular opacity score for octylphenoxypolyethoxyethanol and SDS, whereas with BAC, the opacity score was more predictive of eye irritation than the permeability score. Therefore, a systematic investigation of opacity and permeability measures with surfactants using this approach may be helpful with elucidating MIE #2 of the AOP. In addition, information on the potential of a substance to cause skin irritation (*e.g.*, OECD TG 439 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2020</Year><RecNum>14808</RecNum>

<DisplayText>[101]</DisplayText><record><rec-number>14808</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044884">14808</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Reconstructed Human Epidermis Test Method, In vitro Skin Irritation</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>26, https://www.oecd-ilibrary.org/docserver/9789264242845-en.pdf?expires=1596045726&id=id&accname=guest&checksum=2580E92A5C889D0DD65599260E7866D3</pages><volume>439</volume><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>]) and/or skin corrosion (*e.g.*, OECD TG 431 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2019</Year><RecNum>14809</RecNum><DisplayText>[102]</DisplayText><record><rec-number>14809</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596044976">14809</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>In Vitro Skin Corrosion: Reconstructed Human Epidermis (RhE) Test Method</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>29, https://www.oecd-ilibrary.org/docserver/9789264264618-

en.pdf?expires=1596045820&id=id&accname=guest&checksum=E3EE55CBAAFAF0432EAD109F1B39ECF0</pages><volume>431</volume><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>]) *in vitro*, can provide supporting evidence of the potential for a substance to cause similar irritant or corrosive effects in respiratory tract cells. Corrosion effects mediated by pH extremes should be distinguished from necrosis effects *via* membrane disruption, demonstrated by DDAC that causes tissue effects in inhalation studies despite having a neutral pH value of 6.8-6.9 [ADDIN EN.CITE <EndNote><Cite><Author>Sigma-Aldrich</Author><Year>2020</Year><RecNum>14810</RecNum><DisplayText>[103]</DisplayText><record><rec-number>14810</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596045132">14810</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sigma-Aldrich</author></authors></contributors><titles><title>Safety Data Sheet, Product name: Didecyldimethylammonium chloride, Version 8.1, Revision Date: 03/28/2020, Print Date: 05/29/2020</title></titles><pages>9, https://www.sigmaaldrich.com/MSDS/MSDS/DisplayMSDSPage.do?country=US&language=en&productNumber=34466&brand=SIAL&PageToGoToURL=https%3A%2F%2Fwww.sigmaaldrich.com%2Fcatalog%2Fproduct%2Fsial%2F34466%3Flang%3Den</pages><dates><year>2020</year></dates><urls></urls></record></Cite></EndNote>].

CLEs

Several *in vitro/ex vivo* assays may determine whether a new chemical substance is acting *via* the proposed surfactant AOP and can be used to assess chemicals within the Surfactant Category.

For general cytotoxicity ([REF _Ref46931271 \h * MERGEFORMAT]), cell lines are available that are known to be sensitive to the effects of surfactants. The BALB/c 3T3 NRU cytotoxicity test has been reviewed and recommended by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) for use prior to conducting animal testing [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The surfactants with known inhalation toxicity (*e.g.,* octylphenoxypolyethoxyethanol, oleoyl sarcosine, DDAC, or BAC) should be tested in parallel with the new chemical substance to benchmark the results, thereby providing reliable results for estimating the potential for surfactants to cause irritation and cytotoxicity.

OLEs

Based on the results of the testing on the CLEs, given the limitations of the assays, it may be necessary to perform more robust testing. The discussed assays measure single cell types, whereas human and animal airway epithelia are composed of multiple cell types that each have specialized functions. Several human airway models have been developed that allow for the assessment of multiple endpoints in 3D culture systems. Two commonly employed systems are EpiAirway™ and MucilAir™ developed by MatTek Life Sciences and Epithelix, respectively.

Organotypic airway epithelial cultures, such as EpiAirway™ and MucilAir™ are more physiologically relevant than *in vitro* cell lines [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2018</Year><RecNum>14811</RecNum><DisplayText>[105]</DisplayText><record><rec-number>14811</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr"

timestamp="1596045320">14811</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Issue Paper: Evaluation of a Proposed Approach to Refine Inhalation Risk Assessment for Point of Contact Toxicity: A Case Study Using a New Approach Methodology (NAM)</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>33, https://ntp.niehs.nih.gov/ntp/about_ntp/sacatm/2019/september/bcgnd-1-epa_case_study.pdf</pages><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>]. These organotypic cultures, unlike single cell lines, take on a pseudostratified morphology; develop tight junctions; differentiate into multiple cell types, including basal cells, ciliated cells, and goblet cells; generate mucus; exhibit ciliary beating; have xenobiotic metabolizing capacity; and maintain cultural homeostasis for months. Because of these characteristics, these human airway models are expected to better represent the response of *in vivo* tissue to surfactant exposure than cell line cultures of a single cell type. Depending on the anatomical area in the respiratory system where the site of contact/exposure is predicted to occur, using for example RDDR or multi-path particle dosimetry (MPPD) modeling for determining deposition, different 3D cell culture systems are available that are composed of the different cell types that occur at different anatomical sites in the respiratory tract. MucilAir™ provides 3D co-culture models of cells from nasal, tracheal or bronchial sites, as well as a co-culture of cells from small airways (SmallAir™). EpiAirway™ is composed of a co-culture of normal human

tracheal/bronchial epithelial cells, and EpiAlveolar™ is a 3D co-culture model of the air-blood barrier produced from primary human alveolar epithelial cells, pulmonary endothelial cells, and fibroblasts (with and without macrophages).

Exposure of respiratory tract 3D co-culture models to aerosols at the air liquid interface (ALI) using an *in vitro* exposure system, such as those available from Vitrocell® Systems, provides an exposure more comparable to real-life scenarios for inhaled aerosols, although it is a lower throughput compared to *in vitro* two-dimensional exposure systems. Dilution in medium and interaction with medium components does not occur in the ALI exposure systems as in submerged culture systems. The respiratory tract 3D co-culture models are more physiologically relevant due to the fact there is an interaction of the aerosol with a mucus or surfactant layer, as as in humans.

Exposures of these organotypic cultures at the ALI can be combined with other assays for assessing cell function and viability to inform the surfactant AOP elements. Measurement of transepithelial electrical resistance (TEER), LDH-release, and viability assays such as MTT or ATP assays have all been reported for use with these cultures. Further, multiple assays can be performed on the same cultures. TEER measures epithelial integrity, including functionality of intercellular tight junctions. LDH-release measures loss of plasma membrane integrity, which is indicative of cytotoxicity, and MTT and ATP assays measure cell viability. MatTek Life Sciences recommends the MTT assay for use with their EpiAirway™ cultures and recommends the surfactant octylphenoxypolyethoxyethanol at 0.2% concentration as a positive control for cytotoxicity. These assays can also be used to determine an HEC, provided dosimetry models are

available for translation of the internal dose achieved under culture conditions to an equivalent inhalation exposure for the human scenario of interest. Examples of *in vitro* dosimetry models to predict particle doses for submerged cell culture include the *In vitro* Sedimentation, Diffusion and Dosimetry model (ISDD) [ADDIN EN.CITE ADDIN EN.CITE.DATA] and the *In vitro* Sedimentation, Diffusion and Dissolution Dosimetry (ISD3) model [ADDIN EN.CITE ADDIN EN.CITE.DATA].

Significant progress has been made toward achieving the objectives to use high-throughput *in vitro* assays and computational models to evaluate potential adverse effects of chemical exposures [ADDIN EN.CITE <EndNote><Cite><Author>NRC</Author><Year>2007</Year><RecNum>14741</RecNum><DisplayText>[14, 108]</DisplayText><record><rec-number>14741</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596019531">14741</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>NRC</author></authors></contributors><titles><title>Toxicity Testing in the 21st Century: A Vision and a Strategy, Washington, D.C. The National Academies Press</title></titles><pages>216, DOI: https://doi.org/10.17226/11970</pages><volume>ISBNs: Ebook: 978-0-309-13412-5; Paperback: 978-0-309-15173-3</volume><dates><year>2007</year></dates><urls></urls></record></Cite><Cite><Author>NRC</Author><Year>2017</Year><RecNum>14812</RecNum><record><rec-number>14812</rec-number><foreign-keys><key app="EN" db-

id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596045703">14812</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>NRC</author></authors></contributors><titles><title>Using 21st Century Science to Improve Risk-Related Evaluations, Washington, D.C., The National Academies Press</title></titles><pages>200, https://doi.org/10.17226/24635</pages><volume>ISBNs: Ebook: 978-0-309-45351-6; Paperback: 978-0-309-45348-6</volume><dates><year>2017</year></dates><urls></urls></record></Cite></EndNote>]. To translate the effects to higher levels of biological organization, a battery of assays with varying complexity and physiological relevance may be needed. The 3D human airway cell culture systems are available to add evidence to the AOP and increase confidence of the physiological relevance to humans.

Precision-cut lung slices (PCLS) is an additional method to develop OLE data. The PCLS measures multiple endpoints, such as LDH for cytotoxicity and IL-1α for pro-inflammatory cytokine release, in *ex vivo* cultures of rodent and human lung slices, to determine whether a chemical is likely to be toxic to the respiratory tract by inhalation exposure [ADDIN EN.CITE ADDIN EN.CITE.DATA]. PCLS contain intact alveoli, rather than monolayers of one or two cells types (co-cultures). Crucially, in contrast to organoids, cell types are present in the same ratios and with the same cell–cell and cell–matrix interactions as *in vivo* systems. PCLS are often used in toxicological and anatomical studies regarding contractility in relation to asthma and other respiratory illnesses, such as emphysema [ADDIN EN.CITE <EndNote><Cite><Author>Sanderson</Author><Year>2011</Year><RecNum>14814</RecN

um><DisplayText>[110]</DisplayText><record><rec-number>14814</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046031">14814</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Sanderson, M. J.</author></authors></contributors><auth-address>Department of Microbiology and Physiological Systems, University of Massachusetts Medical School, Worcester, MA 01655, USA. Michael.Sanderson@umassmed.edu</auth-address><titles><title>Exploring lung physiology in health and disease with lung slices</title><secondary-title>Pulm Pharmacol Ther</secondary-title><alt-title>Pulmonary pharmacology & therapeutics</alt-title></titles><periodical><full-title>Pulmonary pharmacology & therapeutics</full-title><abbr-1>Pulm Pharmacol Ther</abbr-1></periodical><alt-periodical><full-title>Pulmonary pharmacology & therapeutics</full-title><abbr-1>Pulm Pharmacol Ther</abbr-1></alt-periodical><pages>452-65</pages><volume>24</volume><number>5</number><edition>2011/05/24</edition><keywords><keyword>Animals</keyword><keyword>Cell Physiological Phenomena</keyword><keyword>Disease Models, Animal</keyword><keyword>Humans</keyword><keyword>Lung/pathology/*physiology</keyword><keyword>Lung Diseases/*pathology</keyword><keyword>Microscopy/methods</keyword><keyword>Muscle Contraction/physiology</keyword><keyword>Organ Culture Techniques</keyword></keywords><dates><year>2011</year><pub-dates><date>Oct</date></pub-dates></dates><isbn>1094-5539 (Print)
1094-5539</isbn><accession-num>21600999</accession-

num><urls></urls><custom2>PMC3168687</custom2><custom6>NIHMS296121</custom6><electronic-resource-num>10.1016/j.pupt.2011.05.001</electronic-resource-num><remote-database-provider>NLM</remote-database-provider><language>eng</language></record></Cite></EndNote>]. Therefore, physiological responses, other than cytotoxicity, that may be evoked by the surfactant may be evaluated. One further advantage of PCLS is that the assay can be performed on multiple species to determine inter-species variability in susceptibility.

The PCLS test system has been pre-validated in multiple, independent laboratories, and the results showed correlation with *in vivo* LC_{50} values [ADDIN EN.CITE ADDIN EN.CITE.DATA]. While considered an alternative test, use of rodent tissue still requires use of animals, but when compared to *in vivo* inhalation tests, this assay reduces the number of animals that would be needed to conduct dose response studies. From a rat lung (1 g), approximately 200 slices can be prepared. In general, for each test substance concentration, 2 slices are used, resulting in 100 different concentrations or repeats that can be tested using tissue from a single rat. Additionally, PCLS cultures are stable for up to 4 weeks and allows for exposures *via* liquid media or, with additional adaptations, air. As such, the PCLS system meets the goal of reducing animal testing, although dosimetry models for their translation to HEC are not yet developed. Human PCLS, derived from, for example, rejected but otherwise healthy transplant tissue, can also be used to measure cell/tissue viability, local respiratory inflammation and physiological function. These endpoints can be measured in single and repeated exposures in a metabolically competent system within the normal architecture of the lung in a more relevant model system, replacing the need for animal testing [ADDIN EN.CITE ADDIN

EN.CITE.DATA]. Mechanistic rodent and human PCLS studies may be conducted in parallel to understand species specific difference in toxicological effects. The rationale for selection of the PCLS assay, as with any inhalation toxicity assay, should be scientifically justified in advance of initiating testing.

Uncertainties/Limitations of the Surfactant AOP Approach

A number of *in vitro* assays have been discussed as to their potential utility within the context of surfactant AOP elements (*i.e.*, MIEs, CLEs, and OLEs). Uncertainties and limitations associated with these assays are discussed for each of the above testing systems, as well as others [ADDIN EN.CITE ADDIN EN.CITE.DATA], it is important to consider that these assays were not systematically tested using surfactants or benchmarked against *in vivo* inhalation toxicity data on surfactants using traditional test method validation approaches. Nonetheless, these assays, alone or in combination should be considered to provide information on whether a new chemical meets the Surfactant Category criteria and/or to understand whether the new chemical may be more or less bioactive or toxic than the sub-category comparator chemicals. EPA will generally use the framework and analogue toxicity data identified in this investigation to assess potential risks from surfactants.

In this regard, approaches to evaluate the scientific confidence of test methods for hazard assessment and risk assessment continues to evolve. A fit-for-purpose framework, employing specific criteria to establish relevancy, reliability, variability, sensitivity, and domain of applicability for evaluating a new method to inform specific decisions has emerged from the regulatory science community to address the challenges posed for validation of NAMs [ADDIN

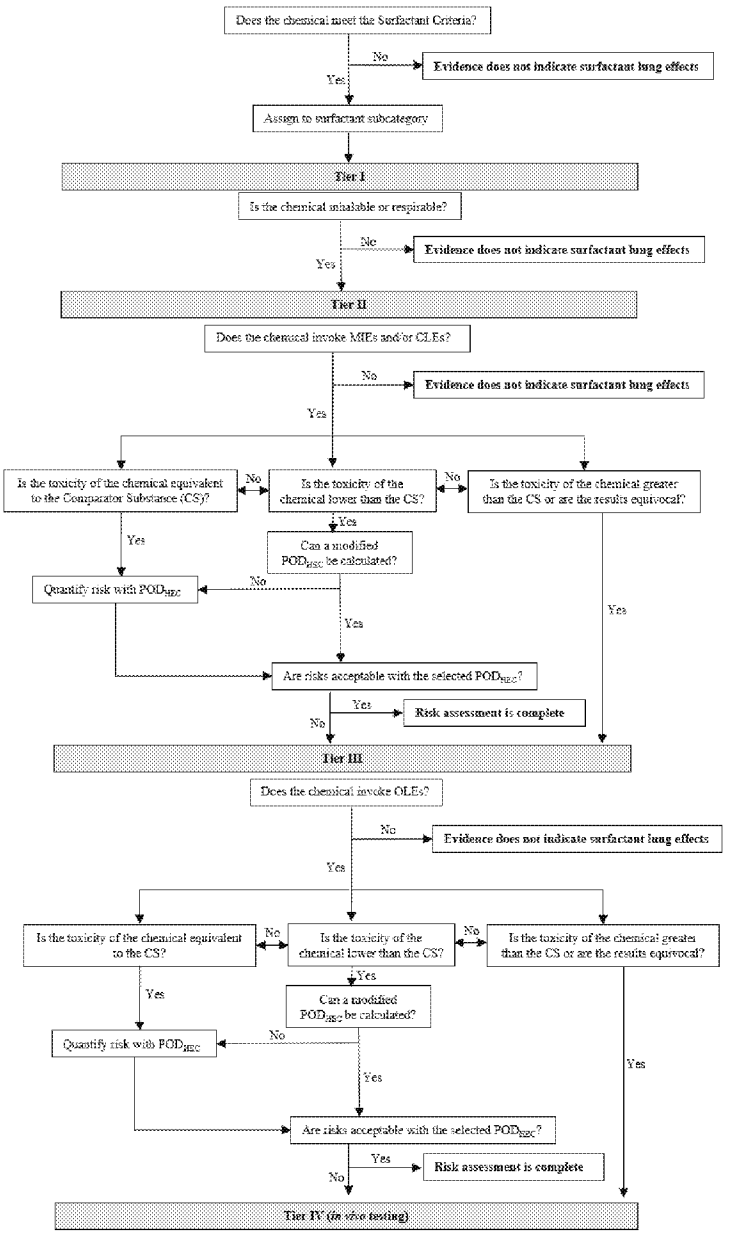
EN.CITE ADDIN EN.CITE.DATA]. Such fit-for-purpose validation approaches are intended to be flexible and adaptable and to provide data sets, prediction analysis results, inference models, *etc*. in a transparent manner that enable other scientists to confirm the performance of the assays and inference models, as well as evaluate the rationale for using these assays in a specific decision context.

Once such fit-for-purpose scientific evaluations are documented, there are several ways that these assays can be used to reduce and replace animal testing. First, testing can be performed based on the surfactant AOP to evaluate the potency of new surfactants versus a comparator substance within the relevant subcategory that has repeated exposure inhalation toxicity data. Second, depositional data using models such as RDDR or MPPD for determining the depositional fraction of the new surfactant may be used for test concentration estimation and for estimating a potency ratio. Finally, *in vitro* to *in vivo* extrapolations (IVIVEs) may be used to determine a HEC for quantitative risk assessment.

Tiered-testing Strategy

The first step in the tiered-testing strategy is to determine if the evaluated substance meets the Surfactant Criteria. If so, then assign the substance to the appropriate surfactant subcategory (nonionic, anionic, or cationic) and determine whether any of the representative subcategory chemicals may serve as an acceptable toxicological analogue for risk assessment or as a comparator substance for tiered testing. If a representative subcategory chemical is determined to be an acceptable toxicological analogue to the new chemical substance, then quantify risks using the toxicological analogue. If the MOE is equal to or greater than the benchmark MOE, then

tiered testing is not required on the new chemical substance. If the MOE is lower than the benchmark MOE or if a determination cannot be made on whether any of the representative subcategory chemicals are acceptable toxicological analogues, then proceed with tiered testing using the most appropriate subcategory chemical as a comparator substance to the new chemical substance. As detailed below, the tiered-testing strategy commences with the least complex, most efficient testing methods, and at each subsequent tier, the complexity of the test system increases, commensurate with the hypothesized surfactant AOP, to more effectively emulate the biology and physiology of the *in vivo* respiratory tract system. It is envisioned that both the new chemical substance and the comparator substance will be evaluated side-by-side in the NAM assays. The results of these studies may lead to the conclusion that the comparator substance is an acceptable toxicological analogue to the new chemical substance. Alternatively, the results may support that higher tiered testing is warranted, particularly when the new chemical substance has ~~lower or~~ higher toxicity than the comparator substance. If *in vivo* testing is conducted, it may not be necessary to run the comparator substance in the *in vivo* tests, given that suitable inhalation studies are available on the comparator substances. A summary of the proposed tiered-testing strategy is summarized in [REF _Ref48210489 \h * MERGEFORMAT] and discussed further below.



Scheme **[SEQ Scheme * ARABIC]**. Proposed tiered-testing strategy for general surfactants.

Tier I—Physicochemical properties

Surfactants are proposed to cause a specific sequence of biological events in the respiratory tract if they are inhaled. Manufacture, processing, or use of a surfactant in an inhalable form, (*i.e.*, ≤ 100 μm aerodynamic diameter) is therefore, an initial consideration of the potential for a surfactant to cause toxicity to the respiratory tract. Particle size is an established parameter for determining inhalability/respirability of particles/droplets. Several validated test methods exist for determining potential inhalability/respirability, *i.e.*, particle size, of a new chemical substance (*e.g.*, OECD GD 39 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>2018</Year><RecNum>14819</RecNum><DisplayText>[114]</DisplayText><record><rec-number>14819</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046851">14819</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Guidance Document on Inhalation Toxicity Studies, Series on Testing and Assessment, No. 39 (Second Edition)</title><secondary-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</secondary-title></titles><periodical><full-title>Environment Directorate, Joint Meeting of the Chemicals Committee and The Working Party on Chemicals, Pesticides and Biotechnology, Organization for Economic Cooperation and Development</full-title></periodical><pages>106, https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2009)2

8/rev1&doclanguage=en</pages><volume>ENV/JM/MONO(2009)28/REV1</volume><dates><year>2018</year></dates><urls></urls></record></Cite></EndNote>], ISO 21501-1:2009 [ADDIN EN.CITE <EndNote><Cite><Author>ISO</Author><Year>2009</Year><RecNum>14820</RecNum><DisplayText>[115]</DisplayText><record><rec-number>14820</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596046993">14820</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>ISO</author></authors></contributors><titles><title>Determination of particle size distribution — Single particle light interaction methods — Part 1: Light scattering aerosol spectrometer</title></titles><pages>https://www.iso.org/standard/42728.html</pages><volume>ISO 21501-1:2009</volume><dates><year>2009</year></dates><urls></urls></record></Cite></EndNote>], OECD TG 110 [ADDIN EN.CITE <EndNote><Cite><Author>OECD</Author><Year>1981</Year><RecNum>14821</RecNum><DisplayText>[116]</DisplayText><record><rec-number>14821</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596047114">14821</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>OECD</author></authors></contributors><titles><title>Particle Size Distribution/Fibre Length and Diameter Distributions; Method A: Particle Size Distribution (effective hydrodynamic radius); Method B: Fibre Length and Diameter

Distributions</title><secondary-title>OECD Guidelines for the Testing of Chemicals</secondary-title></titles><periodical><full-title>OECD Guidelines for the Testing of Chemicals</full-title></periodical><pages>13, https://www.oecd-ilibrary.org/docserver/9789264069688-en.pdf?expires=1596047951&id=id&accname=guest&checksum=A9C13F0DFDCF2A5DD4DD39DAC64C47BC</pages><volume>110</volume><dates><year>1981</year></dates><urls></urls></record></Cite></EndNote>], and OPPTS 830.7520 [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1996</Year><RecNum>14822</RecNum><DisplayText>[117]</DisplayText><record><rec-number>14822</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596047315">14822</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Particle Size, Fiber Length, and Diameter Distribution</title><secondary-title>Product Properties Test Guideline, Office of Pollution Prevention and Toxics, U.S. Enviornmental Protection Agency</secondary-title></titles><periodical><full-title>Product Properties Test Guideline, Office of Pollution Prevention and Toxics, U.S. Enviornmental Protection Agency</full-title></periodical><pages>13, https://www.regulations.gov/contentStreamer?documentId=EPA-HQ-OPPT-2009-0151-0030&contentType=pdf</pages><volume>EPA 712-C-96-037</volume><dates><year>1996</year></dates><urls></urls></record></Cite></EndNote>]). The studies shown in Table 3 suggest that the total respiratory tract may be affected from surfactants; therefore, inhalable forms ($\leq$ 100 μm) were identified as the most relevant for quantitative inhalation risk assessment. As a practical matter, a particle size cutoff of greater than

1% inhalable particles/droplets by weight (wt%), determined in a well conducted study using a valid measurement method will generally be considered as triggering a quantitative assessment of inhalation toxicity on a new chemical substance meeting the Surfactant Criteria. EPA will generally assess the potential inhalation toxicity for a new surfactant chemical substance when the manufacture, processing or use results in greater than 1% (by weight) of the surfactant particles/droplets having a particle size of less than 100 μm. This wt% cutoff is consistent with EPA's "trace amounts" threshold for the nonreportable content for nanoscale materials [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2017</Year><RecNum>14823</RecNum><DisplayText>[118]</DisplayText><record><rec-number>14823</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596047488">14823</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Chemical Substances When Manufactured or Processed as Nanoscale Materials; TSCA Reporting and Recordkeeping Requirements</title><secondary-title>Federal Register</secondary-title></titles><periodical><full-title>Federal Register</full-title></periodical><pages>3641-3655</pages><volume>82</volume><number>8</number><dates><year>2017</year></dates><urls></urls></record></Cite></EndNote>].

If inhalable particles/droplets can be generated at greater than 1 wt% during manufacturing, processing, or any of the uses for the new chemical substance, proceed to Tier II.

[PAGE]

Tier II—*In vitro/Ex vivo* studies

The following *in vitro/ex vivo* test methods may provide potentially useful information to determine whether a new chemical substance invokes MIEs and CLEs. In order to determine the best approach for *in vitro/ex vivo* testing, a pre-notice consultation with EPA is highly encouraged ~~given that, for surfactants, none of the following studies have been validated using the traditional interlaboratory round robin method to determine lung effects/toxicity~~. In general, the testing approach in this tier should include a combination of assays, such as one that measures MIE #1 (*e.g.*, epithelial lining fluid/cell perturbation or pulmonary surfactant interaction/loss of function), one that measures MIE #2 (*e.g.*, cell membrane disruption/interaction/penetration), and one that measures CLEs (*e.g.*, loss of membrane integrity/general cytotoxicity) (see [REF _Ref46931271 \h * MERGEFORMAT]). *In vitro/ex vivo* eye irritation studies may also demonstrate cell interaction or penetration and general cytotoxicity.

For each assay, the comparator substance for the respective subcategory of surfactants should be tested under identical conditions. Further, the particle size distribution data may be used with dosimetry models such as RDDR model or the MPPD model to aid with identifying the regions in the respiratory tract where deposition is expected to occur and the appropriate test concentrations for the *in vitro/ex vivo* test systems, considering for example the surface area of the culture system or *ex vivo* tissue, loss mechanisms, *etc.*

Notwithstanding the uncertainties with the above assays, each may be used to determine a starting point to calculate a modified POD_{HEC} using *in vitro* to *in vivo* extrapolation (IVIVE) for

the purpose of evaluating the relative potency of the new chemical substance versus the comparator substance. Several investigations have provided insight on approaches for accomplishing this, although with different assay systems [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In doing so, a weight of scientific evidence evaluation should be performed considering the structural features, physicochemical properties, and assay results on the new chemical substance versus the comparator substance. Based on this evaluation, the most biologically relevant endpoint(s) should be used to calculate a POD. BMD modeling may be applied to derive a $BMCL_{1SD}$ metric, as a possible metric, although the metric of one standard deviation should be used with caution [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>2019</Year><RecNum>14825</RecNum><DisplayText>[120]</DisplayText><record><rec-number>14825</rec-number><foreign-keys><key app="EN" db-id="sp9w2fxejsw0zre0azr5evearxfds0err5sr" timestamp="1596048386">14825</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>EPA</author></authors></contributors><titles><title>Transmittal of Meeting Minutes and Final Report for the Federal Insecticide Fungicide and Rodenticide Act, Science Advisory Panel (FIFRA SAP) Meeting held on December 4 and 6, 2018</title><secondary-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</secondary-title></titles><periodical><full-title>Office of Chemical Safety and Pollution Prevention, U.S. Environmental Protection Agency, Washington, D.C. 20460</full-title></periodical><pages>51,https://www.regulations.gov/contentStreamer?documentId=EPA-HQ-OPP-2018-0517-0030&contentType=pdf</pages><volume>EPA-HQ-OPP-2018-